Validation of Digital Pathology In a Healthcare Environment

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Abstract

Digital pathology is a dynamic, image-based environment that enables the acquisition, management and interpretation of pathology information generated from a digitized glass slide. The Digital Pathology System (DPS) includes a whole slide scanner (WSS), image acquisition software, image viewing and database software, image analysis software, and the necessary IT infrastructure to support the DPS. Validation is an ongoing process to establish documented evidence that provides a high degree of assurance, that a process or system will consistently perform according to predetermined specifications and quality attributes. To date, efforts to validate digital pathology systems in a clinical healthcare environment have been very limited. Therefore the strengths and weaknesses of validation are essentially unknown. Documentation from governing organizations such as the Food and Drug Administration (FDA), Centers for Medicare and Medicaid (CMS), and the College of American Pathologists (CAP) around validation practices is scarce. Attempts at validation of digital pathology systems are likely limited due to a lack of understanding on how to efficiently conduct a validation and how to navigate regulations that could have an impact on laboratory accreditation and healthcare compliance.

Validation of a digital pathology system (DPS) is necessary for clinical use to ensure laboratory compliance for CLIA, State regulations, The Joint Commission, and CAP accreditations, to protect patient safety and confidentiality, to assure digital pathology data is accurate, and to maximize the value of a DPS. This white paper will not provide a step by step approach to validation. It will serve as a high level overview of what should be considered, while aligning with other resources available to the pathology community including CAP Checklists and the recently published CAP draft guidelines for “Validating Whole Slide Imaging Systems for Diagnostic Purposes in Pathology.” (1)

This white paper will use a key code to help readers naviagate the information based on their role in validation.

P	Pathologists
L	Laboratory managers & histology personnel
IT	Hospital and pathology department information technology personnel
Q	Quality Assurance, Quality Control, Regulatory personnel

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Introduction

Efforts to validate digital pathology systems in a clinical healthcare environment to date have been very limited. Documentation from governing organizations such as the Food and Drug Administration (FDA), Centers for Medicare and Medicaid (CMS), and the College of American Pathologists (CAP) around validation practices is scarce. Attempts at validation are likely limited due to a lack of understanding on how to efficiently conduct a validation and how to navigate regulations that could have an impact on laboratory accreditation and healthcare compliance.

Pathology laboratories are familiar with the required programs for quality assurance (QA) and quality control (QC) programs, however labs may or may not be familiar with validation practices of laboratory developed tests, or non FDA approved medical devices. This paper will not provide a step by step approach of validation, and will look at familiar QA/QC practices and how they should be considered when validating digital pathology. This paper will serve as an overview of what is needed and what should be considered during validation, while aligning with other resources available to the pathology community including the CAP Checklists and the recently published CAP guidelines for "Validating Whole Slide Imaging Systems for Diagnostic Purposes in Pathology." (1)

Digital Pathology

Digital pathology, as defined by the Digital Pathology Association, is a dynamic, image-based environment that enables the acquisition, management and interpretation of pathology information generated from a digitized glass slide. It is used worldwide in drug development, reference lab, hospital, and academic medical center settings. Applications include education, research, image analysis, archival and retrieval, LIS/LIMS integration, secondary consultations, and virtual slide sharing.

Overview

Why validate digital pathology?

Validation is an ongoing process to establish documented evidence that provides a high degree of assurance, that a process or system will consistently perform according to predetermined specifications and quality attributes. Validation of a digital pathology system (DPS) is necessary for clinical use to ensure laboratory compliance for CLIA, State regulations, The Joint Commission, and CAP accreditations, to protect patient safety and confidentiality, to assure digital pathology data is accurate, and to maximize the value of a DPS. CAP draft guideline #1 states,

> "**All institutions or practices considering the implementation of WSI technology for clinical diagnostic purposes must carry out their own validation study."**

A digital pathology system should be validated based on it's intended use, and the solutions usefulness concerning specific applications. Digital pathology applications may include but are not limited to primary diagnosis, image analysis, consultations, quality assurance, and interoperative diagnoses. CAP draft guideline #2 states,

> **"Validation for each diagnostic application is necessary (e.g. reading frozen section slides, reviewing immunohistochemistry slides, etc.). WSI should not be used for clinical purposes other than the one validated, unless separate validation for that purpose is undertaken."**

Validation of a DPS will create a baseline for acceptable performance and use of whole slide imaging scanners, data management software, and image analysis in a healthcare laboratory environment.

A Successful Validation

A successful outcome for the validation of your digital pathology system will be management approval for the intended use of the DPS and to maintain your lab accreditations with the DPS in place. Validation of a DPS is not easy. The validation process must be taken very seriously, and requires the ability to monitor and control changes to the DPS on an ongoing basis. A strong foundation can assist in the execution of a DPS validation project. Characteristics of a strong foundation for a DPS validation include:

- An anatomic pathology specimen tracking system (i.e. barcodes) from accession, through sign-out, and archival of blocks and slides.
    - Every slide should have a unique ID
- Experience validating laboratory developed tests (LDT) and/or other digital imaging devices for clinical use (i.e. Cytogentics, FISH systems) that may or may not be FDA approved
- Procedures for data retrieval and preservation of whole slide images
- The laboratory applies all current ASCO/CAP recommendations[2,3] and current CAP checklist guidelines to all methods used to create stained slides, and has conducted the appropriate verification and/or validation required.

The Validation Project

The validation of a DPS involves a series of test and production activities where the opportunity for interjection of human fallibilities' are enormous. Defects, or an undesired result, may begin to occur at the very inception of the validation process; where the objectives of the DPS may be erroneously or imperfectly specified. There is further risk during the design and development stages when these objectives are mechanized. Therefore all validation efforts, when possible should be conducted in a test environment, a controlled area that will not inadvertently affect patient care, laboratory accreditation, or healthcare compliance. A test environment will provide a proof of concept, create a foundation to validate against, and will allow for a smooth transition into the anatomic pathology workflow.

The validation process is a project, a task carefully planned and designed to achieve a goal. Before validation begins, take the time necessary to prepare for your validation project. Often the intended use and other important information has been identified and outlined in a request for information (RFI) or request for proposal (RFP) during the DPS acquisition process. If you have this information, incorporate it into the DPS validation project. Proper preparation will mitigate risk and create a foundation for success. A well designed project will provide a business case, have an opportunity and goal statement, establish a clear scope, and outline the responsibilities of individuals involved. See the Appendix for an example of a validation project summary.

Business Case

The business case should provide a summary of the validation process, and describe why validation is important, and why validation of the DPS should be a priority in your laboratory.

Opportunity & Goal Statements

The opportunity statement should describe what problems will arise if validation of the DPS is not addressed. It will reinforce the business case but be more specific. The opportunity statement should answer what the problems are, how big the problem is, and what the impact of the problems will be on the laboratory or the DPS.

The goal statement will compliment the opportunity statement by describing what will be accomplished, give a measurable target for desired results, and state a projected completion date to reach the goal.

Scope

Determining the scope of the validation is a very important step in the project. Scope will define the boundaries of what will be and will not be achieved by this validation, identify constraints, and list any assumptions. To define the scope consider the following:

- What is the intended use of the DPS?
- How you will test for the intended use?
- What will you scan? i.e. type of slides, specimens, etc.
- What are the limitations of the DPS?
- What is out of scope for the intended use of the DPS?

Responsibilities

The validation project will be overseen by a few, however the results will have an impact on many. Validation efforts should be led by a team of individuals from a variety of backgrounds which include:

- Pathologists
- Histology Personnel
- Hospital or pathology department IT personnel
- QA/QC group members

Each team member's responsibilities and role on the team should be clearly outlined and available to the entire team. Individual members must agree to their responsibilities and take ownership of their role on the validation team.

Validation of a Digital Pathology System

Validation of a Digital Pathology System involves a series of quality assurance (QA) test and production activities that will demonstrate and produce documented evidence, to endorse with a high degree of assurance, the Anatomic Pathology laboratories intended use for digital pathology. All QA activities must continue throughout the life of the DPS because of human interjection into to a Quality Management Plan.

Although the CAP draft guidelines does not require validation of each individual component of a DPS, this white paper will discuss many of these individual components and discuss what areas should be reviewed and monitored throughout the validation process.

Acceptance Criteria

Acceptance of the DPS endorses the reliability of the DPS. Reliability is a quality control measurement and with established specifications provides a quantitative criterion for Digital Pathology System performance. To capture the essence of reliability is to provide insight to real time performance of the system, which in turn could affect budget, workflow, personnel, and patient care. This is especially important when interoperability's are established between software applications to increase efficiencies within the workflow process.

Hardware

Hardware includes the digital pathology scanner and the computer, including the monitor, if separate from the scanner. For the validation of hardware this paper will make the following assumptions:

- Hardware must live within the proper environmental conditions as specified by the hardware manufacturer. Examples of environmental conditions include temperature, humidity, and work surface.

Scanner

The scanner is a medical device that provides the means to create a digital image output file, known as a Whole Slide Image (WSI) from a glass slide. What is then monitored for reliability is the mechanical function of the scanner, and the clarity of the WSI the scanner outputs. Examples of what could be monitored or validated for mechanical function of the scanner include;

- Uninterruptible Power Supply (UPS)
- Luminance (light intensity)
- Chromaticity (Color temperature)

Routine, internal audits are recommended to evaluate the ongoing performance of the scanner. Examples of what could be routinely audited during operation are;

- WSI quality
- Mechanical hardware issues
- Environmental conditions
- WSI storage space

Computer

The computer operates the software application designed for scanner. Minimal requirements are made available by the vendor, but optimal requirements should be explored and documented because of the heavy usage on the computer by the scanner and its complemented application. If images will be reviewed at the computer monitor, then monitor specifications are also extremely important for image evaluation. Examples of what could be monitored or validated within the initial computer are,

- Location
- Resolution
- Power
- CPU
- Graphics card
- Network connectivity
- Fire wire/Ethernet ports

Examples of audits during operation are,

- Computer performance
- Software performance
- Connectivity to network/peripherals
- Overall stability/reliability

Software

There are different vendor solutions for scanner software and its applications. Software function is accomplished by following the manual set forth by the vendor and testing it for proper usage and configuration. There are four aspects of the scanner application software 1) directing the scanner modes, 2) monitoring the scanner mechanics, 3) the control of output files, also known as a WSI and 4) scanner performance.

Viewer Software

A viewer application can be an extension of the scanning software, data management software, or run independently on a client desktop from a web browser. Viewer software often contains additional tools that aid in easy review of the WSI, can aid in clinical diagnosis, or provide additional information. Examples of these tools are algorithm analysis for IHC, cellular architecture, color distribution, measurement tools, digital zoom, annotations, etc. A validation of this is easily accomplished by following the vendor expected outputs and functionality provided in the user manuals. Audit

functions should include previously output or known values that can be reused to assess continuous linearity and calibration of viewer tools.

Data Management Software

Data management is a key component when relying on fast and timely retrieval of information associated with a digital pathology case. This technology allows the transference of information across gradient lines between the APLIS and DPS systems. A person testing this should follow their vendors guidelines as well as guidelines for transference of information from one source to another.

In the absence of a current CAP Digital Pathology checklist, the following CAP Lab General checklist [4] items may assist in the validation of the data management software:

- **GEN.45500 Interface Encoding/Transmission Phase I**
- **GEN.46000 Reference Range/Units Transmission Phase I**
- **GEN.47000 Interface Security Phase II**
- **GEN.48500 Interface Result Integrity Phase II**
- **GEN.43088 LIS Integrity Phase II**

Validation of software performance should be administered through a set of control images (based on vendor specs and site specific slides), an image overlay (phantom components, area of interest, field of view capture), and a wedge or test slide (luminance, color).

EMR/APLIS

Prior to validating the Anatomic Pathology Laboratory Information System (APLIS) integration the following assumptions are made:

- The reference to APLIS encompasses both the Electronic Medical Record (EMR) and APLIS
- Review the Digital Pathology Association white paper entitled “Interoperability between Anatomic Pathology Laboratory Information Systems and Digital Pathology Systems” found at http://digitalpathologyassociation.org/dpa-white-papers.
- Consult with your IT department and APLIS support staff for reference to the integration testing between an APLIS and other equipment already performed
- Contact your DPS or APLIS vendor to understand the data being transferred between the two systems.
- All Standard Operating Procedures should be updated with all changes either to the APLIS or DPS system.
- Support Procedures should be updated to include information such as vendor contacts, downtime procedures when the integration link has been compromised and steps that your IT staff can take to support the integration

In the absence of a current CAP Digital Pathology checklist, the following CAP Lab General checklist [4] items may assist in the validation of the APLIS integration:

- **GEN.45500 Interface Encoding/Transmission Phase I**
- **GEN.48500 Interface Result Integrity Phase II**
- **GEN.50057 Slide/Image ID Phase II**
- **GEN.50614 Clinical Information Access Phase I**
- **GEN.51171 Telepathology Appropriate Use Phase I**

Integration between an APLIS and a DPS can vary between Dual System Metadata Sharing and APLIS-Integrated Digital Pathology (both methods are described in detail in the white paper listed above, “Interoperability between Anatomic Pathology Laboratory Information Systems and Digital Pathology Systems.”) With both integrations the APLIS is the authoritative source of the case information and the DPS is the authoritative source of the digital image and its associated metadata. Depending on the type of integration that the site has implemented between their APLIS and DPS, the data validation may differ.

Validation of Dual System Metadata Sharing

With this integration, the primary system for managing imaged cases is the DPS. Workflow consists of the following:

- Patient, case and histology data is sent from the APLIS to the DPS at various event triggers throughout the specimen lifecycle.
- When the slide is scanned and if there is a unique identifier, the DPS will automatically match the scanned image with the case.
- The pathologist primarily uses the DPS to manage his case list, create his “virtual” piles and view the imaged slides.
- The DPS can query the APLIS for other information on the current case and also prior case information to assist the pathologist in his case review.
- If there are any annotations and quantitative analyses performed on the DPS, this data can then be transferred back to the APLIS to be stored for reporting purposes.
- All report generation remains in the APLIS system.

Validation of APLIS-Integrated Digital Pathology

With this integration, the primary system for managing cases is the APLIS. Workflow consists of the following:

- The pathologist continues to manage his case list in the APLIS.
- The pathologist would launch the DPS from the thumbnail or URL provided from the DPS.
- If there are any annotations and quantitative analyses performed on the DPS, this data is transferred back to the APLIS to be stored for reporting purposes.
- All report generation remains in the APLIS system.

When developing the validation protocol for integration the following workflows should be evaluated for inclusion. All data outlined in the agreed upon integration specification should be verified for accuracy and that the data is displayed in the required areas of the DPS. All trigger events in the APLIS that can add patient, case, and histology information should be verified.

- **Patient, Case and Histology Information -** The APLIS pushes to the DPS the patient, case, and histology information.

- **Unique Slide Information** - The APLIS can generate a barcode that contains unique case information. The DPS scanner digitizes the glass slide and decodes the barcode. The DPS uses this number to automatically match the slide to the previously entered case (Dual System Metadata Sharing) or uses the unique ID sent from the DPS to match the scanned image to the case in the APLIS.(APLIS Integration Digital Pathology)

- **Digital Image Viewing** - The pathologist now views the digital case within the DPS.

- **Processing Data and Reports** - Annotations and quantitative analyses can be implemented in the DPS. There may be results and/or data outputs from these computer assisted applications that the site may want to transfer to the APLIS from the DPS. Also, there may be Field of Views (snapshots) that were captured and desired for patient reports.

Audit trail- validating the capture of the data

The audit trails associated with the APLIS should be verified to contain the data outlined in the agreed upon integration specification.

In the absence of a current CAP Digital Pathology checklist, the following CAP Lab General checklist [(4)] items may assist in the validation of the APLIS audit trails:

- **GEN.41303 HIPAA Phase II - The laboratory complies with HIPAA.**
- **GEN.41304 Patient Data Accessibility Phase II - There is a documented protocol in place to ensure that patient data are accessible only to those healthcare personnel who are authorized to review test results.**
- **GEN.43800 Data Input ID Phase II - There is an adequate system to identify all individuals who have entered and/or modified patient data or control files.**

Backup and archival

Glass slides must be stored, typically for around seven years, based on your state guidelines. Today, there are no archival regulations in place concerning WSI (with exception to FISH), and the archival of the WSI is at the discretion of the clinical laboratory (e.g. laboratories can delete the WSI after use). [5] Validation of servers where WSI are stored should fall within the CAP guidelines, as well as your individual institutions policies for testing of your storage and retention. The Information technology Department is a good place to start for internal IT requirements for testing. Back-up should be tested according to your institution guidelines and regularly checked for performance.

In the absence of a current CAP Digital Pathology checklist, the following CAP Lab General checklist [4] items may assist in the validation of any backup and archival solutions;

- **GEN.48750 LIS Interface Shutdown/Recovery Phase II**
- **GEN.44150 Error Message Response - System Back-up Phase II**
- **GEN.44200 Unscheduled Downtime Phase II**
- **GEN.43946 Data Preservation/Destructive Event Phase II**
- **GEN.43933 Data Storage Capacity Monitoring Phase I**
- **GEN.43088 LIS Integrity Phase II**

Image life cycle management is determined by ones institution and departmental guidelines for daily/workflow usage. How long the image is available within a system can vary depending on one's usage and need. Please refer to your IT departmental procedures as well as ones need for image retention.

Validation of Specific Digital Pathology Applications

The majority of specific information on validation in this portion of the paper is being derived from the recently released CAP draft guidelines "Validating Whole Slide Imaging Systems for Diagnostic Purpose in Pathology." [1] It is important to understand that prior to validation of an application, validation of the DPS should take place and the following should be addressed:

- Standard operating procedures and policies on WSI systems are in place
- Training, education and competency for all staff involved including histology personnel, pathologists, etc
- Routine Instrument service and maintenance procedures
- Procedures for data retrieval and preservation

Other factors and requirements influencing, such as CAP Checklists, similar technologies will be discussed also in an attempt to give the user additional information that may be helpful when implementing this technology in the clinical setting.

Primary Diagnosis

There are CAP checklist requirements for telepathology. Telepathology is defined as electronic, multimedia communication between pathologists for the purpose of primary diagnoses and diagnostic consultation second opinion [6], and one of the modes for telepathology is virtual slides or whole slide images. The CAP Telepathology checklist applies to the following uses:

- **Primary diagnosis made by telepathology**
- **Frozen section diagnosis**
- **Formal second-opinion consultations**
- **Ancillary techniques in which the pathologist participates in interpretation of images**

Therefore the conclusion can be drawn that the Telepathology checklist would apply to a DPS for primary diagnosis. The Telepathology checklist does not cover validation of the WSI system but the Cytopathology checklist [7] could under the following requirement;

- **CYP.05257 Implementation/Validation Protocol**

This requirement covers the implementation and validation of new instruments in the cytology laboratory and includes automated screening instrumentation which are similar in technology to a DPS and therefore this guideline may also be applicable to a DPS.

This also brings up recently published information [8,9] on how current regulations (FDA and CLIA) cover clinical laboratories that incorporate WSI systems for use in diagnostic services. WSI systems are medical devices subject to FDA authority. Currently WSI approval is limited to HER2/neu, and ER/PR analysis via 510(k) system-level clearance[2]. There are unanswered questions with respects to the FDA and premarket review of WSI systems through the 510(k) process and whether or not the FDA will choose to regulate these devices. If the FDA chooses not to regulate these devices then they would potentially fall under CLIA in the category of laboratory-developed test (LDT) and therefore subject to CLIA validation requirements for LDT's[8].

Validation of digital image evaluation compared to glass slide evaluation (Concordance of digital and glass slide evaluation) for primary diagnosis. There are some issues to consider in the validation of WSI for routine diagnostic applications.[8]

- Separating the device from the practitioner
- Pathologist experience
- Washout and validation setting
- Types of data generated
- Measuring accuracy
- Measuring bias
- Measuring precision (intra-rater, inter-rater and inter-instrument)
- Sample size
- Generalizability of findings

Below is a summary of the recent CAP draft guidelines [1] which address most of the validation concerns listed above. It covers 13 basic statements. Please note these are draft guidelines and therefore are subject to change:

Statement 1. All institutions or practices considering the implementation of WSI technology for clinical diagnostic purposes must carry out their own validation.

Statement 2. Validation for each diagnostic application is necessary. WSI should not be used for clinical purposes other than the one validated.

This will require a separate validation study for each clinical application for which WSI is being used and therefore WSI can only be used for applications that have been validated. A list of clinical applications could be, but may not be limited to the following:

- Primary routine surgical pathology diagnosis with H&E only
- Primary routine surgical pathology diagnosis including special stains, immunohistochemical (IHC) stains, and In-situ hybridization (ISH)
- Primary routine non-gynecological diagnosis
- Frozen section diagnosis
- Formal second opinion consultations
- Specific ancillary techniques such as image analysis

Statement 3. The validation study should closely emulate the real-world environment.

The goal of any validation is to conduct the validation in a manner that mimics how WSI will be used in the specific laboratory work environment. The "test or validation environment" should be the same as the "go live environment." Careful consideration of the following parameters need to be addressed:

- Scan magnification
- Turnaround time
  - For example since time to frozen section diagnosis is important the time to scan and view the digital slide should be including in a validation study for frozen section diagnosis

- Report turnaround times for routine, special studies, etc

Statement 4. Validation of the entire WSI system should be performed and it is not necessary to separately validate each individual component.

A WSI system may consist of the following different components:,

- Scanner
- Hardware
- Software
- Network
- Data storage and archive system
- Viewing monitor

Although the CAP guideline above does not require validation of all components this decision of what to validate and what not to validate is ultimately up to the laboratory.

Statement 5. A pathologist adequately trained to use the WSI system must be involved in the validation process.

The validation of WSI systems need to include all individuals involved in the process from the pathologist to other laboratory staff such as laboratory managers, histology personnel, IT personal and operators. All need to be adequately trained prior to the validation process. Vendors can facilitate the training process.

Statement 6. Validation of WSI systems should involve specific types of specimens and their preparations, but not specific tissues, diseases, microscopic changes or diagnosis.

Each type of sample preparation should be validated separately (e.g. fixed verses frozen tissue) but validation is not required for specific tissue types, diseases or microscopic changes.

Statement 7. The validation process should include a sample set of approximately 100 cases that reflect the spectrum and complexity of specimen types and diagnoses likely to be encountered during routine operation.

This can be accomplished by selecting a consecutive series of cases; there should be lack of bias when cases are being selected.

Statement 8. Digital and glass slides should be evaluated in random order to minimize order effect.

Both the order in which the cases are presented and the order of the modalities (glass vs. digital) used should be randomized.

Statement 9. A washout period (see definitions) of approximately 3 weeks should occur between viewing digital and glass slides.

Statement 10. The validation process should ensure that all the material present on a glass slide, or purposefully selected area(s) on a slide to be scanned, are included in the digital image.

All relevant material on a glass slide that is needed to make a diagnosis is present in the digital image.

Statement 11. Measureable outcomes should establish diagnostic concordance between digital and glass slide for the same observer.

The purpose of the validation process is to measure the differences (outcome) between making a diagnosis with digital slides compared to glass slides and not to determine diagnostic accuracy.

Statement 12. Approval of WSI systems should be limited to the conditions under which validation occurred. Re-validation is requires whenever a significant change is made to any component of the WSI system.

If there are significant hardware or software changes then the DPS would need to be revalidated. There is no definition for what is considered a “significant” change. Significant changes may or may not include software patches, new software

or firmware versions, hardware upgrades or maintenance preformed on the DPS. Ultimately, the decision will be up to the laboratory to determine when they should revalidate, however if the change could potentially affect the interpretation of digital slides, the validation process should be repeated with the new changes incorporated.

Statement 13. Documentation should be maintained recording the methods, measurements and final approval of validation for the WSI system to be used in the clinical laboratory.

In addition to the CAP draft statements there are some additional preferences for validation.[2]

- Measure intra-observer bias and precision
- Use general pathologist with defined device experience
- Require high quality display
- Enriched case sample – stack with difficult cases
- Washout period > 2 weeks
- Analyze each parameter separately (e.g., tumor type, tumor grade)
- 80% power to detect 10% difference in bias or precision
- Generalize all surgical pathology except hematology and cytology

Image Analysis

Digital image analysis is defined by CAP as the computer-assisted detection or quantification of specific features in an image following enhancement and processing of the image, including DNA analysis, morphometric analysis and Fluorescent In-situ Hybridization (FISH). In the clinical setting image analysis has been used for very specific applications such as ER/PR and HER2 testing. Analysis for these particular prognostic markers have undergone much scrutiny resulting in published recommendations through the American Society of Clinical Oncology (ASCO) and CAP and the new CAP checklist guidelines governing this type of testing.

It is not the purpose of this paper to discuss the validation and/or verification process of the staining methods associated with a particular type of analysis. It will be assumed that the user applies all current ASCO/CAP recommendations[2,3] and current CAP checklist guidelines to all methods used in creating the stained slide(s) and has performed the appropriate verification and/or validation required. This is covered in the following CAP Anatomic Pathology checklist [10] under Digital Image Analysis;

- **ANP.23004 – Pre-analytic Documentation**
- **ANP.23006 – Test System Validation**

In the CAP Anatomic Pathology Checklist [10] under Digital Image Analysis also address, calibration, quality control and specimen analysis. Ongoing calibration is required and the following guidelines are associated with that;

- **ANP.23009 Calibration**
- **ANP.23014 Calibration Materials Labeling**
- **ANP.23016 Calibration Materials**

The extent of verification and/or validation required for a particular image analysis system or protocol is dependent upon whether the particular algorithm has been 510(k) cleared by the FDA for that DPS vendor, the stain on which the clearance was issued, and if the algorithm remains unmodified. Some recommendations on validation are as follows:[4]

1. If the laboratory has an assay (previously verified/validated) already in clinical service (used on ≥200 cases) they can use the results of those previous analyzed cases in their validation study when there is a major change to the test system. (e.g. changing analysis methods from manual to automated).
   (A) Sample size should consist of >40 positive cases and >40 negative cases
   (B) Agreement must be >90% for positive result and >95% for negative results

2. For commercial “closed” systems, the vendor provided validation information must be verified by the laboratory.[3] For initial test verification of an unmodified FDA-cleared, FDA-approved assay the following would apply;
   (A) Sample size should consist of >20 positive cases and >20 negative cases
   (B) Agreement must be >90% for positive result and >95% for negative results

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Regulation and Governing Agencies

Governing Agencies

The Anatomic Pathology laboratory is accredited by regulatory and governing agencies that have set forth standards for quality management systems. Even though the new technology of Digital Pathology is just being introduced into the clinical process, the laboratory should continue to follow their existing quality management protocols with this new technology.

The laboratory should create their intended use statement for the Digital Pathology Systems. Based on the current quality management system, an evaluation should occur to determine which regulations apply to the DPS. The Standard Operating Procedures should be updated with these references.

Food & Drug Administration (FDA)

The FDA currently regulates the manufacturers of the medical device and not the laboratory that is using the medical device in their facility.

It is not uncommon for a laboratory to ask the DPS vendor to explain their quality management process and the standards their quality program references. Examples of these may be the following:

- **21 CFR 820 - Title 21 Code of Federal Regulations Part 820 (Quality System Regulation)** - Provides framework of basic requirements for manufacturers. http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/ucm126252.htm
- **21 CFR Part 11 Title 21 Code of Federal Regulations Part 11 (Electronic Records/Electronic Signatures)** - This regulation sets forth the criteria under which the agency considers electronic records, electronic signatures, and handwritten signatures executed to electronic records to be trustworthy, reliable, and generally equivalent to paper records and handwritten signatures executed on paper. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm
- **General Principles of Software Validation; Final Guidance for Industry and FDA Staff.** This guidance outlines general validation principles that the Food and Drug Administration (FDA) considers to be applicable to the validation of medical device software or the validation of software used to design, develop, or manufacture medical devices http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm085281.htm
- **Draft Guidance for Industry and Food and Drug Administration Staff.** This guidance is intended to assist (1) sponsors who are planning to develop a therapeutic product that *depends on* the use of an in vitro companion diagnostic device (or test) for its safe and effective use and (2) sponsors planning to develop an in vitro companion diagnostic device that is intended to be used with a corresponding therapeutic product. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm262292.htm

College of American Pathology (CAP)

If the laboratory is currently regulated by CAP then the laboratory should incorporate the intended use of the Digital Pathology System into their current process and procedures.

The current checklists that you need to review are the following.

- Cytopathology Checklist [7]
- Laboratory General Checklist [4]
- Anatomic Pathology Checklist [10]

The Joint Commission

If the institution is already being inspected by The Joint Commission, the DPS process should be added to the tracer methodology currently defined for your laboratory. The tracer methodology follows the patient documentation from the doctor's order into the lab and back out to the patient reports, including pre-analytic, analytic and post-analytic process with in the lab. The DPS is part of the analytical process and should be added to the tracer methodology. Review Tracer Methodology 101 when evaluating how the DPS will fit in your lab tracer.

The Joint Commission Accreditation Program covers all CLIA specialties and is deemed by CMS.

Health & Human Services

If your laboratory is currently following HIPAA (The Health Insurance Portability and Accountability Act) then the laboratory should incorporate the intended use of the Digital Pathology System into these processes and procedures.

State Law

Refer to your state regulations through the state department of health and human services for validation guidelines around Laboratory Developed Tests.

Center for Medicare and Medicaid Services

There a no specific CLIA regulations for Digital Pathology. Current CLIA certified laboratories, who have already implemented CLIA Histopathology requirements in to the laboratory process should review the following sections to incorporate the intended use of the DPS into CLIA requirements. (5)

- **Section 493.1105: Retention Requirements**
- **Section 493.1251: Procedure Manual**
- **Section 493.1252: Test Systems, Equipment, Instruments, Reagents, Materials and Supplies**
- **Section 493.1253: Establishment and Verification of Performance Specifications**
- **Section 493.1254: Maintenance and Function Checks**
- **Section493.1255: Calibration and Calibration Verification Procedures**
- **Section 493.1256: Control Procedures**
- **Section 493.1291: Test Report**

Regulatory Agencies outside of the United States

For those laboratories outside of the United States, contact the regulatory agencies to determine if there are other checklists that need to be implemented. Check with the vendor to determine if they have received any other clearances, standards or regulations relating to those specific markets.

Examples are:

- CE Mark – European Market
- Canadian Provincial Regulations
- Licensing by Health Canada

A Vendors Role & Responsibilities in Your Validation

A Vendors Role

In this section we assume the term “vendor” also refers to a distributor, or manufacturer of a DPS. The vendor should disclose to the lab what they can and cannot do to support the validation of the DPS for clinical use. Due to the FDA’s regulation of the vendor, the DPS vendor may only be able to support the validation of the DPS based on the specifications and performance requirements set forth in their marketing literature or user guides, or in some cases for applications in which they have received FDA clearance such as HER2, ER/PR image analysis. A current list of all clearances is in the appendix. Also, the FDA website has a database of all 510(k) clearances. Search vendors for their clearances and intended use at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm. Other clinical intended uses (i.e. primary diagnosis) will likely have to be validated by the laboratory on their own since the vendor cannot support non-FDA cleared intended use of a DPS at this time.

At the time of installation, the vendor should have a process to install and implement the system. Typically, the installation is performed in phases and with each phase there is a checklist that certain milestones and criteria has been met. After the vendor has completed their implementation (which includes installation of the hardware and software, training, and integration (if applicable), the site will sign-off and accept the DPS.

A Vendors Responsibility

Vendors have a responsibility to manufacture robust, reliable digital pathology hardware and software solutions and to provide adequate support and training to customers of the DPS.

DPS users should feel welcome to ask vendors about their regulated manufacturing process and to understand what standards of quality and care are put into the manufacturing of a DPS. Vendors manufacture their solutions under standards from the International Standards Organization (ISO). Here are a few examples:

- **ISO – 13485 - Medical Devices - Quality Management Systems - Requirements for Regulatory Purposes.** This International Standard specifies requirements for a quality management system where an organization needs to demonstrate its ability to provide medical devices and related services that consistently meet customer requirements and regulatory requirements applicable to medical devices and related services.
- **ISO 14971 - Application of risk management to medical devices.** This International Standard specifies a process for a manufacturer to identify the hazards associated with medical devices, including in vitro diagnostic (IVD) medical devices, to estimate and evaluate the associated risks, to control these risks, and to monitor the effectiveness of the controls.

Manufacturers and distributors are regulated by FDA's Quality System regulations for medical product distributions in the U.S. Although the process in which a vendor manufacturers their DPS is not part of the validation process, it can give insight into the reliability and consistency of a DPS system. Additional information on the regulated manufacturing process can be found under the Regulatory and Governing Agencies section.

Vendors are also responsible for providing all current, updated manuals, or user guides that apply to the purchased DPS. DPS users should review and become familiar with all documentation that is supplied with the DPS. Use this documentation as a reference when creating and executing your validation plan. During installation of the DPS, the vendor should work together with the site to ensure that all specifications and requirements identified throughout the purchase process are met. If a problem exists, a vendor has a responsibility to resolve problems in a timely fashion. During the purchase process, the site should have an agreed upon response time that has been negotiated with the vendor to resolve issues that are preventing the site from preforming to the agreed upon specifications and requirements.

P L Q

QA & QC Considerations for after Validation

The laboratory accreditation programs mandate that all laboratories have a quality assurance (QA) program. Laboratory QA programs typically have five components; three addressing the test cycle (pre-analytic, analytic, and post analytic) and two addressing global measures of turn around time and customer satisfaction. Quality assurance (QA) monitor data should provide a picture of the level of performance, and ensure the requirements are being continuously met always, every day.

The basic QA factor for a DPS is that the system performs specific functions in the manner the DPS manufacturer intended it to do. The laboratory should pay close attention to how the DPS matches their own intended use, and document all undesirable results including workflow mismatches, image quality concerns, security and audit trail deficiencies. Depending on the volume, frequency, and severity of undesirable results during QA testing, the laboratory must document and make a recommendation on the reliability of the DPS and whether to accept the DPS system. When the DPS is accepted, if there are still some objectionable results, the laboratory should document their mitigation steps or the inability to use the product as specified for an intended use. To achieve a high level of QA, the DPS must not contain a high number of defects in implementation as well as being void of any errors. The DPS must be validated, contain adequate verification, and have audit steps built into the process as defined by the vendor. This information will be incorporated into the customer's current or future Standard Operating Procedures.

In the absence of a current CAP Digital Pathology checklist, the following CAP Lab General checklist [4] items may assist in the need to assess QA/QC with a Digital Pathology system;

- **GEN.13806 Documented QM Plan Phase II**
- **GEN.20262 QM Trends/Corrective Action Phase I**
- **GEN.20316 QM Indicators of Quality Phase II**
- **GEN.20348 Pre-Analytic QM Phase II**
- **GEN.43800 Data Input ID Phase II**
- **GEN.52285 Telepathology QM Phase I**
- **GEN.43920 Multiple Analyzer ID Phase I**
- **GEN.43044 Software Modification Tracking Phase II**

Initial quality assurance checks for reliability of a DPS are established by review of the WSI. Once a WSI is generated by a DPS, the WSI are then transferred to a viewer which can either be a stand alone or connected to a dedicated DPS data management solution, or an APLIS which is another point of engagement for the user. Either way the initial scan must be reviewed for concordance or defective components before release to the active clinical environment. The laboratory must determine the responsibilities and reporting relationships within a QA/QC environment.

Examples of verification or audits are to monitor image issues, monitor issues dealing with mechanical artifact, and to monitor output file identification if shared with LIS. Examples of verification audits for scanning software are; Tissue and cellular structure, color intensity, image resolution, personnel performing audit, image noise, and area of interest algorithm for accuracy.

P L IT Q

Conclusion

Digital pathology is an evolving technology that is very new to the clinical healthcare environment but can have a positive impact on pathology and patient care. To insure a positive impact, a well thought out validation must be conducted. Validation is a complex task, and with any new technology there is a learning curve. As with any validation project, there must be support and commitment from department and laboratory leadership and staff to be successful. Once a baseline validation has been accomplished, the data and validation plan should be documented and available for review. Moving forward the DPS validation plan will need to be monitored and periodically reviewed for changes.

Definitions

TERM	DEFINITION
Digital Image Analysis	Defined by the College of American Pathologists as computer-assisted detection or quantification of specific features in an image following enhancement and processing of the image, including DNA analysis, morphometric analysis and FISH.
Digital Pathology	Defined by the Digital Pathology Association (DPA) as a dynamic, image-based environment that enables the acquisition, management and interpretation of pathology information generated from a digitized glass slide.
Digital Pathology System (DPS)	An image-based computer system that enables the acquisition, management and interpretation of pathology information generated from a digitized glass slide.
FDA Approved	When FDA review is needed prior to marketing a medical device the FDA will "approve" the device after reviewing a premarket approval (PMA) application that has been submitted to FDA.
FDA Cleared	When FDA review is needed prior to marketing a medical device the FDA will "clear" the device after reviewing a premarket notification, otherwise known as a 510(k) (named for a section in the Food, Drug, and Cosmetic Act), that has been filed with FDA.
Intended Use	The method or manner in which a product is used on a daily basis within the laboratory.
Laboratory Developed Test (LDT)	A test developed within a clinical laboratory that has both of the following characteristics: is performed by the clinical laboratory in which the test was developed and is neither FDA cleared nor FDA approved.
Quality Assurance	Quality Assurance, defined by the US Department of Defense directive 1972, is a planned and systematic pattern of all actions necessary to provide adequate confidence that material, data, supplies, and services conform to established technical requirements and achieve satisfactory performance.
Quality Control	Is the ability to detect, reduce, and correct deficiencies in a laboratory's internal analytical process prior to the release of patient results and improve the quality of the results reported by the laboratory.
Quality Management Plan	A document that defines the specific steps that a laboratory will take to ensure that quality is being maintained.
Standard Operating Procedure (SOP)	A written document or instruction detailing all steps and activities of a process or procedure within the laboratory.
Telepathology	Electronic, multimedia communication between pathologists for the purpose of primary diagnoses and diagnostic consultation second opinion.
Validation	An ongoing process to establish documented evidence that provides a high degree of assurance, that a process or system will consistently perform according to predetermined specifications and quality attributes.

TERM	DEFINITION
Washout Period	The time interval between viewing the same case and/or slide using a different (glass or digital) modality
Whole Slide Image (WSI)	A digitized histopathology glass slide that has been created on a slide scanner. The digitized glass slide represents a high-resolution replica of the original glass that can then be manipulated through software to mimic microscope review and diagnosis. Also referred to as a virtual slide.
Whole Slide Imaging	The acquisition process of creating a virtual slide or whole slide image (WSI) on a slide scanner.
Whole Slide Scanner (WSS)	The hardware and associated software required to generate a whole slide image.

Appendix

Validation Project Summary Example

Project Title:

Business Case: The business case should provide a summary of the validation process, and describe why validation is important, and why validation of the DPS should be a priority in your laboratory.

Example: *Validation of our digital pathology system is required for CAP and CLIA accreditation of our laboratory, to obtain reimbursement on tests, and to maintain our high standards for quality patient care.*

Opportunity Statement: The opportunity statement should describe what problems will arise if validation of the DPS is not addressed. It will reinforce the business case but be more specific. The opportunity statement should answer what the problems are, how big the problem is, and what the impact of the problems will be on the laboratory or the DPS.

Example: *If validation of our digital pathology system is not preformed our laboratory will loose money by not being able to preform #___ number of tests and could loose revenue of $_____; we will not be able to provide same day turnaround on our #____consultations per year, and we will loose are ability to be successful with digital pathology and obtain the benefits we purchased the system for.*

Goal Statement: The goal statement will compliment the opportunity statement by describing what will be accomplished, give a measurable target for desired results, and state a projected completion date to reach the goal.

Example*: We will validate our digital pathology system based on our intended use, using the CAP Checklists and Guidelines, by June 30.*

Project Scope: Scope will define the boundaries of what will be and will not be achieved by this validation, identify constraints, and list any assumptions.

Example: *Validation of the digital pathology system will be based on our intended use. Our intended use is to enable pathologists to preform quantitative IHC (CPT 88361) analysis and obtain reimbursement for these tests; to scan all slides being sent out for consultation and to preform same day consultation reviews with specialized pathologists through review of the WSI. All of this must be achieved while maintaining our laboratory accreditation with CAP & CLIA. Validation will not include any applications regarding education, resident training, archival of slides, or slides scanned for use in pathology conferences, or other use cases that do not affect patient care.*

Preliminary Plan	Actual Date	Target Date	**Notes:** Capture lessons learned
Start Date:			
Milestone 1:			
Milestone 2:			
Milestone 3:			
Milestone 4:			
Milestone 5:			
Completion Date:			

FDA 510(k) Approvals for Digital Pathology [5]

Company	Date	510(k) Number	Tissue, Stain	Reagent	Application
Aperio	8/2009	K080564	Breast-HER2, neu	Dako	Tunable Image Analysis
Aperio	10/2008	K080254	Breast, PR	Dako	Manual read on a monitor
Aperio	08/2008	K073667	Breast- ER/PR	Dako	Image Analysis
Aperio	12/2007	K071671	Breast, HER2/ neu	Dako	Manual read on a monitor
Aperio	10/2007	K071128	Breast-HER2/ neu	Dako	Image Analysis
Applied Imaging (purchased by Danaher/Leica)	03/2004	K012138	Breast- ER/PR	Dako	Image Analysis
Applied Imaging (purchased by Danaher/Leica)	01/2004	K031715	Breast-HER2/ neu	Dako	Image Analysis
Bioimagene (purchased by Roche/Ventana)	10/2010	K092333	Breast- P53/ Ki-67	Dako	Image Analysis
Bioimagene (purchased by Roche/Ventana)	02/2009	K080910	Breast-HER2/ neu	Dako	Image Analysis
Bioimagene (purchased by Roche/Ventana)	02/2007	K062756	Breast-HER2/ neu	Dako	Image Analysis, software only
Cell Analysis	12/2003	K031363	Breast-ER	Dako	Image Analysis (software only)
Clarient/ Chromavision (purchased by ZEISS)	02/2004	K012138	Breast- ER/PR	Dako	Image Analysis
Clarient/ Chromavision (purchased by ZEISS)	12/2003	K032113	Breast-HER2/ neu	Dako	Image Analysis, System

Company	Date	510(k) Number	Tissue, Stain	Reagent	Application
Tripath (purchased by Roche/Ventana)	09/2006	K062428	Breast- p53	Ventana	Image Analysis
Tripath (purchased by Roche/Ventana)	04/2006	K053520	Breast- Ki-67	Ventana	Image Analysis
Tripath (purchased by Roche/Ventana)	08/2005	K051282	Breast, HER2/ neu	Ventana	Image Analysis
Tripath (purchased by Roche/Ventana)	05/2005	K050012	Breast- ER/PR	Ventana	Image Analysis

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