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Digital Cytopathology Quality Guidelines

Quality Assurance & Laboratory Standards Committee
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1.0 Purpose and Scope

This document provides guidelines for veterinary digital cytopathology quality and training for pathologists who wish to maintain patient safety and high quality data generation and interpretation. It specifically targets digital image capture from glass slides and cytopathologic interpretation in the digital setting. Histopathology and hematopathology are out of the scope of these guidelines, though relevant published references (e.g., digital pathology validation and statistical evaluation) are cited and discussed. Artificial intelligence has been implemented in other clinical pathology areas, and the authors agree that AI should meet similar standards for noninferiority; however, the use of AI in digital cytopathology is beyond the scope of these guidelines. The guidelines are not intended to be all inclusive or prescriptive; rather, they provide a minimum standard for digital imaging using scanners with associated case submission software, and static images when used to provide a cytologic interpretation. Digital cytopathology is a rapidly advancing field, and while the current body of evidence remains scarce and inconsistent in quality, it is summarized in this document. The intended audience includes veterinary pathologists and residents, laboratory technologists and technicians, and associated staff who scan slides or perform other duties to implement and use digital cytopathology.

This guideline considers the following quality assurance aspects of veterinary digital cytopathology:

a. Preanalytical Factors
    i. Sample and Accessioning Requirement
    ii. Training of Operators
b. Analytical Factors
    i. Important Aspects of Scanners and System
    ii. Validation of Scanners and Systems
    iii. Personnel Training
c. Postanalytical Factors
    i. Considerations and quality assurance for reporting

2.0 Definitions

Color calibration: For the purposes of this guideline, the complex mechanisms by which digital imaging systems ensure that the colors of scanned specimens are equivalent when viewed digitally on a computer monitor and with a microscope.[1]
Concordance: Measurement of agreement of different methods, which may be between observers (interobserver) or using the same observer (intraobserver).[2] Specifically, it is defined as agreement between diagnoses or interpretations when glass slides or digital cytopathology is used.[3]
Digital Pathology: A dynamic, image-based environment that enables the acquisition, management, and interpretation of pathology information generated from a digitized glass slide.[4]
Discordance: Disagreement between diagnoses or interpretations.[3]
Equivalence: The extent to which Whole Slide Images represent the contents of a glass slide, often assessed as intraobserver concordance. This differs from non-inferiority in that equivalence infers that digital cytopathology is not unacceptably different from glass slides while non-inferiority infers that digital cytopathology is not unacceptably worse than glass slides.[5-7]
Gamma correction: Or gamma, is a feature of digital images that defines the relationship between a

pixel's numerical value and the brightness of the surface. It corrects the differences between how a camera captures content, how the monitor displays it, and how the eye processes the resultant image.[8]
High resolution: Display of cellular details with great clarity and precision, often achieved by a digital scanner through high pixel density and magnification.
Non-inferiority: The concept that digital cytopathology is not worse than glass slides by a predefined margin (i.e., the diagnoses are acceptably concordant). Intraobserver variation between light and digital microscopy is compared at multiple time points. All potential quality issues with glass slides exist with digital cytopathology and may lead to error.[3,6]
Operators: Laboratory personnel who manage the scanning and quality control of digital slides.
Personnel: For the purposes of this document, operators, in addition to veterinary pathologists and residents who perform interpretation and diagnosis.
Point of Care (POC): Instruments or analyses that reside outside of the traditional clinical pathology reference laboratory.[9] In the context of this guideline, it refers to slide scanners that reside in an in-clinic laboratory.
Preliminary Interpretation: Communication of cytologic findings that precedes the full/finalized cytopathology report, with the intent of providing initial impressions.
Reference Laboratory: Private, state, or university diagnostic or research pathology laboratory.
Region of interest (ROI): A limited area of the sample/digital image of specific clinical concern.
Resolution: The amount of detail in an image, the size of the pixel array, or the minimum distance by which two objects can be separated and still appear distinct.[10,11]
Sharpness: The level of detail and clarity in an image, or conversely, the degree of blur. Many image viewers offer the ability to adjust sharpness after an image is captured (post-capture adjustment).
Static image: A still image taken of a region of a slide at a single magnification. Often captured using a camera attached to an ocular, or externally (cell phone).[12]
Stitching: Collating of multiple smaller digital image frames, either by lines or tiles to create a dynamic WSI.[11,13]
Telecytopathology (Telepathology): For the purposes of this document, telepathology is the practice of remote pathology using telecommunication links to enable electronic transmission of digital pathology images. Telepathology can be used for remote rendering of primary diagnoses, second opinion consultations, quality assurance, education, and research.[14] Other sources in the literature have defined telepathology/telecytopathology slightly differently, using it to describe remote-controlled live microscopy which may be used in rapid onsite evaluation (ROSE).[15]
Tinctorial quality: For the purpose of this guideline, the staining properties of microscopic structures, indicating how well they absorb and retain dyes, which affects visibility and contrast in microscopic analysis. This applies to the color composition of both glass slide microscopy and digital images.
Turnaround time (TAT): TAT can be defined in several different ways depending on the workflow and context (i.e., reference lab samples vs point of care). For the purpose of this guideline:
Case TAT: time that elapses between patient sampling and the laboratory's report transmission to the clinician.
Submission TAT: time from the receipt of the case at the laboratory to a completed report.
Pathologist TAT: time it takes for a pathologist to review a case from opening the accession to finalizing their report, measured and optimized by some laboratories.
User interface (UI): The software platform where the submitting clinician/technologist uploads the WSI and enters the appropriate supporting documents and clinical information, and where pathologists view the WSI and enter their cytologic findings. This is often web-based.
Validation: Confirmation, through the provision of objective evidence, that the requirements for a

specific intended use or application have been fulfilled. Focus: Fitness for purpose or suitability for intended use.[16] Process of determining error associated with a candidate instrument/method in order to determine if the amount of error is acceptable for the intended use of the test.[17]
Verification: Confirmation, through the provision of objective evidence, that specified requirements have been fulfilled. Focus: Conformance to requirements, e.g., a scanner's performance that is consistent with manufacturer claims.[16] Demonstration that a validated method achieves the established performance characteristics in the user's hands according to the manufacturer's claims for the method's specifications (i.e., performance as intended).[17]
Whole slide image (WSI): A conventional glass slide is robotically scanned to generate a complete digital reproduction that can be transmitted electronically and viewed on a computer screen. Using this technology, the pathologist can navigate the sample at different magnifications, emulating "conventional light microscopy in a computer generated manner".[13]
Zoom: Increasing the size of a region of interest in a visual field. This is analogous to increasing the microscope objective magnification when viewing a glass slide.
Digital zoom**:** A software-based manipulation of previously captured pixel data that mimics the effect of an optical zoom. Digital zoom can result in a loss of picture quality, resolution, and details.
Optical zoom**:** The glass elements of the microscope objective physically move to enlarge the image, which does not result in a loss of image quality.
Z-stacking (AKA **extended depth of focus** or **focus stacking**)**:** a compilation of images taken between the first and last depths of focus (**z-axis**) that are then merged into a single digital image file, which mimics the fine focus feature of the glass slide sample on the light microscope. These files are large, and the scanning time is longer.[10,18]

3.0 Background

Digital pathology incorporates the acquisition, management, sharing, interpretation, and reporting of pathology case information, including slides and case data, in a digital environment. Digital pathology images are created when specimens on glass slides are captured with a scanning or imaging device to provide a high resolution digital image that can be viewed on a computer screen or mobile device. Specific modalities include capturing still images of individual fields (static telepathology), regions of interest (ROI), and whole slide imaging (WSI). Additionally, digital pathology systems with remotely controlled microscopes are also used, though less frequently. While digital pathology was initially used primarily for archiving and teaching purposes, the FDA approval of WSI systems for primary diagnostic use for surgical pathology in 2017 marked a pivotal step forward, signaling a growing acceptance and integration of digital pathology into clinical practice.[19,20] The evolution of digital pathology has been marked by significant technological advancements, including improvements in scanning speed, image resolution, and data management systems.[21] Digital pathology has enabled pathologists to work remotely (particularly critical during the COVID-19 pandemic), may accelerate case turnaround time (by reducing the time needed to ship slides to pathologists), improves collaboration between specialists, and opens new avenues for research and education. Currently, most studies of pathologists' efficiency and concordance of digital versus glass slides have used diagnostic histopathology samples.[22-24] Integrating image analysis and artificial intelligence (AI) tools into digital pathology platforms is expected to transform the field further as technology advances.

The Pathology and Laboratory Quality Center for Evidence-based Guidelines of the College of American Pathologists (CAP) released its first guideline on validating WSI for diagnostic purposes (histopathology) in 2013.[14] At the time, they noted a lack of evidence-based guidelines for clinical laboratories to validate the novel technology. In the 2022 revision, CAP collaborated with the American Society for Clinical Pathology and included hematopathology but excluded cytopathology.[3] This revision also used the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach, which many organizations worldwide use as a standard in guideline development.[25] International guidelines for pathologists in the human medical field were also reviewed, including the Royal College of Pathologists of Great Britain (RC Path) guidance on telepathology released in 2013 and best practices for implementing digital pathology in 2018[15], the Royal College of Pathologists Australasia digital pathology guideline released in 2015 and updated in 2020[26], and the European Society of Digital and Integrative Pathology (ESDIP) guideline for the implementation of a digital pathology workflow in the anatomic pathology laboratory in 2021.[27] In 2024, the American Society of Cytopathology (ASC) published a concept paper with recommendations for digital cytopathology and implementation in practice.[28] The quality and training recommendations made in this ASVCP QALS document align with the thoroughly researched CAP, RC Path, and ASC guidelines on digital pathology.

Over the last 20 years, digital pathology in veterinary medicine has expanded significantly with the increasing availability, cost-effectiveness, and performance of high throughput scanners. One study assessed WSI for histopathologic evaluation of canine tumors and found digital slides were non-inferior to glass slides.[29] Multiple digital pathology studies for veterinary cytopathology also support the validity of various digital modalities for both diagnostic and teaching applications.[5,30-32] Starting in the mid-2010s, commercial veterinary laboratories began implementing WSI to scan histologic sections for remote reading by anatomic pathologists globally. From 2017-2021, multiple diagnostic companies introduced cytopathology slide scanning for both reference lab and point of care (POC) applications.[33] As of this writing, nearly all commercial veterinary diagnostic companies have switched to WSI for histopathology, and it is becoming increasingly common for cytopathology. Academic and government diagnostic laboratories and pharmaceutical companies have been slower in adopting WSI or other forms of digital pathology. For a more detailed history of the digital pathology landscape in veterinary medicine, the reader is directed to more comprehensive reviews.[34-38]

The transition to digital pathology has faced obstacles, including the need for significant investment in infrastructure and information technology (IT) resources, operator and pathologist training, proprietary software, lack of a standardized digital format, and regulatory challenges (primarily in human medicine and toxicologic pathology). Scanning cytopathology slides presents more technical challenges than scanning histologic sections, such as limitations to optical resolution, longer scan times, sample thickness and tinctorial quality variations, color calibration, increased storage needs, and server capacity. Digital cytopathology in the POC setting is also more challenging due to the use of different stains (aqueous Romanowsky rapid stains versus alcohol-based Wright Giemsa or May-Grunwald Giemsa), the need to integrate scanning into hospital workflows, operation of equipment by untrained, non-laboratory personnel, and difficulties in accessing large digital files remotely in clinical environments with inconsistent internet connectivity. ROI and static image evaluations have been used as alternatives to WSI in this context to address some of these challenges.

Evaluation of the total cost of ownership (or lease arrangement), including software and hardware, internet requirements, maintenance costs, and upgrades, is necessary and should be balanced with the long term benefits and efficiencies gained from implementing a digital cytopathology system.

The combination of a cost-effective software and hardware system that enhances diagnostic accuracy and improves workflow efficiency may contribute to better patient care and result in cost savings. Peer reviewed published validation and business cases are needed, as minimal data on diagnostic quality and cost effectiveness comparing traditional glass versus digital cytopathology exist in the veterinary field at this time.

As with adopting all new technologies, there is a need to balance the benefits against the associated risks. Unfortunately, comprehensive evidence of the safety of digital pathology in all settings is not yet available. Pathologists should seek to ensure safe clinical practice at all times. We expect technology to continue advancing, and training recommendations may change over time. Therefore, we recommend revising this guideline in five years versus the typical ten years for ASVCP QALS guidelines. At the time of this document, digital capabilities are not present in all training facilities, and glass slide evaluation still predominates in most academic facilities and residency programs.

4.0 Preanalytical Quality Considerations

All preanalytical quality considerations needed to generate an accurate cytopathology interpretation using glass slides are also required for cytologic interpretation using digital pathology. The reader is referred to Section 3 for General Preanalytical Factors and Section 9.1 for Preanalytical Factors for Cytopathology of the ASVCP Guidelines: Principles of Quality Assurance and Standards for Veterinary Clinical Pathology (3.0) which provide detailed preanalytical directions for reference laboratory responsibilities (e.g., client education, glass slide preparation, and sample accessioning).[17]

4.1 Sample Requirements

Sample preparation is critically important to deliver a sample of diagnostic quality for evaluation. Sample source, preparation method, and staining protocols may all impact the ability to interpret the microscopic findings. Because all available slides may not be stained or scanned, the number of slides available (stained and unstained) should be noted in writing and available for pathologist's review, should further evaluation of additional slides be necessary.

Sample location on the slide may be dictated by the scanning apparatus requirements. Most systems cannot scan to all edges of a slide; therefore, centralized placement of the sample (e.g., fine needle aspirates, blood smears, and fluid preparations) will help ensure the entire cellular area can be scanned by the instrument. Placing the sample at least ¼ inch from the edges is generally recommended. Additionally, since many scanning systems struggle with imaging densely prepared smears or lack fine focus without z-stacking technology, it is important to consider the thickness and density of the sample before scanning. Stain type and quality may impact the scan quality and readability of the glass slides. The stain utilized should be clearly communicated to the cytopathologist. Staining protocols may need to be optimized to ensure that samples are neither under- nor overstained, depending on the specific optics of the digital scanner. Digital color and contrast adjustments may be applied to fine tune tinctorial properties to better replicate the sample's appearance using the light microscope. Some WSI scanners require the application of coverslips to achieve optimal resolution. A single coverslip that spans the entirety of the sample must be affixed to the appropriate side of the slide and air bubbles eliminated. Scanner settings may need to be adjusted according to manufacturer's recommendations when affixing a coverslip is optional and both options are used. For samples prepared in a POC setting with rapid Romanowsky stains, an optimized staining protocol should be provided to the client and made readily accessible to ensure optimal quality. Samples may need to be rejected due to poor staining quality at the pathologist's discretion.

Some scanners require slides to be coverslipped, adding another step to the workflow and increasing the potential for user error. Oil or glue may obscure the objective, air bubbles may prevent image capture of the specimen, and inappropriate cassette loading may result in unfocused images. Additionally, permanent coverslips affixed with glue preclude repeat staining or other techniques intended to improve specimen or image quality. Coverslipping may enable cleaning of the slide and archive, which may be preferred. Some facilities may elect to scan non-coverslipped glass slides if resolution appropriate for diagnostic interpretation can be achieved. State laws or regulations appropriate to the location regarding medical records and digital and/or glass archives must be followed, which may contribute to decision making regarding preanalytical procedures.

For generation of static images, it is critical to include images of all diagnostically relevant areas of the slides, ideally at multiple magnifications from low to high.[39,40] Out-of-focus images should not be included in the submission to the pathologist. The number of images submitted/received for review should be included in the submission and reported to the pathologist by the trained operator. Since there are a variety of systems that can be used to capture static images, that information should also be readily available (e.g., cellphone or mounted microscope camera). Additionally, notation of microscope objectives used, a calibrated scale bar, or size reference structures (e.g., RBCs, granulocytes) should be depicted to aid in interpretation. At the pathologist's discretion, submissions may be rejected or interpreted as non-diagnostic or inconclusive if there are insufficient numbers of images taken at varying magnification, poor image quality, or insufficient clinical data.[37]

4.2 Accessioning: Case and Sample Information Provided in the User Interface (UI)

The digital diagnostic case and sample presentation should be equivalent to that provided for glass slide interpretation in the laboratory diagnostic setting. This is particularly important as many pathologists work remotely, often in a different state, province, or country from where the sample was processed. As such, cytopathologists should have access to all information that would otherwise be available onsite. As submission forms routinely contain the name of the submitting clinician in addition to a phone number, email address, and physical address, cytopathologists should have access to this information via the user interface (UI) to facilitate communication. For example, given the global nature of digital cytopathology and the more localized distribution of certain diseases, knowing the patient's geographical location can be crucial in generating a differential diagnoses list. Pathologists should also have access to all pertinent clinical information submitted by clinicians or hospital personnel through the UI. This includes the patient's signalment, lesion location, gross or radiographic description, duration (if known), sample collection method, and associated clinical signs, as these details are essential for meaningful cytologic interpretation. (Table 1). Generally, the pathologist's ability to access all patient data quickly (e.g., CBC, chem, UA, other) will speed interpretation and therefore TAT.

4.2.1 Glass Slide and Sample Data

Hospital or laboratory personnel should provide any pertinent gross and physical characteristics of the glass slide before scanning (e.g., slide appeared greasy, received broken, previously stained) that may influence interpretation, including any information written on the slide that the application of labels may obscure (e.g., lesion location). The UI should have a free text field for entering slide and/or sample related information to enable appropriate communication of these data.

For fluid samples, the gross appearance (e.g., color, turbidity), the details of the preparation method (e.g., direct smears, concentrated preparations, cytocentrifuged preparations), total solids, total protein and other measured solute (e.g., triglyceride, glucose, cholesterol, etc.) concentrations, and cell counts should be provided to the cytopathologist.

Table 1 Recommended Accessioning Data in the User Interface[17]

Patient Information:

Patient and owner name

Patient signalment (species, breed, age, sex, reproductive status)

Submitter Information:

Submitting clinician

Phone number

Email address

Physical address

Lesion Description:

Lesion location

Duration

Gross description and appearance

Diagnostic imaging results

Associated clinical signs

Sample Submission Information:

Gross/physical description of the glass slide

Information written on the slide (possibly under the laboratory's label)

Thumbnail/ low magnification image of the submitted slide

Preparation method (e.g., FNA, direct smear, cytocentrifuged/concentrated preparation, ear swab, etc.)

Type of stain (e.g., Diff-Quik® (Siemens Healthcare Diagnostics) or a comparable rapid stain, modified Wright's stain, etc.)

Fluid Samples:

Refractometer total protein and/or total solids

Gross fluid appearance: color, clarity, turbidity

Total protein and measurement method (if not refractometer)

Total nucleated cell count and counting method

Packed cell volume

Qualitative Assessment:

Quality Assurance information for the sample (e.g., red sample/hemolyzed) (free text box)

Comments (free text box)

4.3 Personnel/Operator Training for Digital Cytopathology Sample Acquisition

The multistep process whereby glass slides are created, converted to WSIs, and transmitted to the pathologist includes sample preparation, staining, data input, and slide scanning. All steps may occur in the in-clinic laboratory, the reference laboratory, or glass slides prepared in the in-clinic laboratory may be sent to the reference laboratory for staining and/or scanning. To minimize TAT and maximize sample quality, all personnel involved in the digital cytopathology process must participate in the requisite training modules for all relevant responsibilities:

- Technical field service representatives should install all scanners and provide initial onsite training for instruments both in the clinic and in the reference laboratory.
- Adequate training and ongoing technical support are essential to utilize software, troubleshoot issues, and maintain updates effectively. Digital cytopathology vendors should offer software training programs, responsive customer support services, and have a comprehensive understanding of the medical records systems that integrate with their product(s). Reference laboratories should have technical support services to assist in-clinic personnel as needed.
- Reference laboratories should provide tutorials for correct slide preparation (smearing and staining of blood, fluid, and tissue cytology samples) in the form of schematics, online videos, and troubleshooting information to reinforce these critical

steps in the sample submission process. Tailoring these training materials to different audiences (e.g., POC versus reference laboratory) may be necessary.
- Reference laboratories should provide proficiency testing or competency assessments to ensure that operators are adequately trained and capable of producing a diagnostic digital sample. Any pathologist using digital cytopathology should request proficiency/ competency assessments of staff if they are not already provided.

Operators capturing static images for the purpose of interpretation by a pathologist should have adequate training. As expected, formal studies have shown that image quality and pathologist interpretations improve when photographers have cytology experience.[41] Diagnostic laboratories that provide static image digital cytopathology services should provide training for their clients through online or hands-on laboratories and/or educational materials (e.g., published videos, handouts). Pathologists should have a way to provide ongoing feedback in the comment sections of their reports or through the UI to aid the submitter or operator in improving image quality.

4.4 Scanning area

Digital scanners may scan the slide in its entirety or use autodetection algorithms to scan smaller regions of the slide suspected to contain cellular material. The entire cellular portion of cytologic preparations should be included in the scanned regions. Manually scanning a small ROI to reduce scan time increases the risk that the scan does not represent the original slide. Unscanned cellular regions may contain important diagnostic material and incomplete scans may not be representative of the sample or lesion, leading to misinterpretation. A macroscopic or subgross image (thumbnail) of the slide should be visible to the pathologist, and the area(s) selected for scanning should be demarcated so that the complete scan of the cellular regions can be assessed. The thumbnail is a critical tool for quality control of submissions and scanning. WSI may be preferred to ensure comprehensive analysis.

If the majority of WSI scans or static images are of suboptimal diagnostic quality (e.g., blurry/out of focus), samples should be rescanned or images should be retaken until a diagnostic sample that can be used for interpretation is generated. Equivalence or non-inferiority to glass slides enables concordant cytopathologic interpretation. If rescanning is unsuccessful, review of glass slides may be considered.

For static images, the submitted images will not depict the entire slide. Personnel capturing images should be trained to take multiple images at varying magnifications in several areas of the slides. Additionally, they should be trained to capture all the various cell populations and structures present on the slide. Glass slide or WSI submission and review may be requested for interpretation at the discretion of the pathologist.

5.0 Analytical Quality Assurance for Digital Cytopathology

5.1 Scanner Hardware Options and Characteristics

It is critical to the success of any cytopathology digitization effort to select the right scanner, associated software, and data storage solution for the facility and integrate them into the laboratory and/or practice workflow. Unlike digital radiography and digital histopathology, digital cytopathology is relatively new in both veterinary and human medicine and, as a result, represents a less mature market from a hardware perspective. One consequence of this immature market is the lack of a single, standardized file format (such as DICOM for radiology); pathology scanners output a range of files from jpeg to tiff, saves, and a variety of proprietary formats that may not be interoperable across different slide viewer and Laboratory Information Management System (LIMS) software systems. More standardized digital formats are expected in the near future which will ease the transition to digital cytopathology. Compatibility with existing and planned laboratory systems should be considered when purchasing a scanner and accessory equipment.

Cytopathology is unique in multiple ways that impact the technology and equipment selected for digitization. First, unlike histopathology, where nearly all tissues are sent to reference laboratories for processing to slides by trained/specialized histology personnel, cytopathology slides are often generated in the clinic, where they may be digitized at the POC, or mailed or courier-delivered to a reference laboratory where they are then stained and scanned. Second, cytologic samples are not fixed planes of tissues cut to a uniform thickness. Rather, they are three-dimensional aggregates of cells and fluids of variable thickness, which can impact the optical requirements of scanning hardware.[42]

Numerous slide scanners capable of digitizing cytopathology samples are currently on the market, and more debut yearly. These scanners vary substantially in price, throughput, scanning resolution, ease of use, and software ecosystem. No single scanner can be recommended to fit all situations for POC and reference laboratory settings. Differences in caseload, clientele, context (POC vs reference laboratory), slide processing, staining, and personnel training may impact the diagnostic utility of any scanner. Therefore, performance criteria are facility dependent. Thus, it is imperative for a laboratory to determine its key requirements in writing before evaluating scanning solutions and then properly validate them as described in this document. The scanning throughput must be adequate for the laboratory's caseload. Scanners should be validated for the intended use and scanner manufacturers' claims should be verified (Section 6).

Marketing materials for scanners may include claims of digitization at a resolution equivalent to a 40-80x microscope objective. However, this may be achieved by using a lower objective with roughly 2x digital zoom created by software adjustments to the image and resolution may be decreased compared to a light microscope objective of equivalent magnification. This is largely because scanning using 20x objectives, many of which are optimized for histopathology, is much faster than using a 40x or higher objective. Very few scanners currently on the market can digitize at 100x objective equivalency. Even when this is possible, the scan times are typically exceedingly long, and file sizes can be hundreds of gigabytes, posing significant file storage and IT challenges. Z-stacking, or scanning at multiple depths, also impacts resolution and allows a pathologist to toggle through multiple planes akin to focusing using a traditional light microscope. While many scanners on the market can provide z-stacking, this creates similar scan time and file size challenges as high-resolution objective scanning. For example, in one veterinary digital cytopathology validation study, the mean scan time for z-stack scanning slides at 7 layers with a 40x objective was 3 hours.[5]

At least 40x magnification equivalent (~0.25 μm/pixel) is recommended for digital cytopathology, recognizing that this may not be adequate for some tissue evaluation, e.g., bone marrow. Despite validating and verifying WSI scanners and selecting an appropriate scanner with current technology, a percentage of slides will likely be non-diagnostic due to suboptimal focus or insufficient resolution for fine details (e.g., chromatin, granules, microorganisms), excluding diagnostically relevant areas of the slide, or simply failing to scan altogether (Section 8.2). Laboratories should have an auxiliary process for identifying and remedying cases that fail digitization through rescanning and/or directing them to a manual glass slide review.

5.2 Software Considerations

Effective implementation of a fully digital user experience in clinical settings requires careful consideration of several factors, including internal IT requirements and identifying key software features essential for clinicians and clinical pathologists. When IT department support is necessary to integrate digital cytopathology, early communication about vendor needs and institutional requirements is crucial before the evaluation process begins. For clinical settings without corporate or institutional oversight, such as privately owned practices, internet security and infrastructure must align with software specifications while protecting client medical records. After all technical requirements are clarified, the software's design and functionality can be appropriately assessed.

Software features have been designed to improve the submission process compared to manual submissions, while maintaining essential processes and redundancies required for appropriate case material management and communication between the submitter and the clinical pathologist. For example, the manually entered information fields for signalment, lesion site, gross description, clinical history, etc., can be left blank on paper submission forms, but in software UI, these fields can be made mandatory for submission. Traditional measures of submission quality control, such as allowing the clinical pathologist to confirm that the sample is associated with the correct patient information, should be incorporated into software interfaces (Table 1). For example, images of glass slide labels can be incorporated into submissions and compared to the patient identifiers provided by the submitter.

Critical software features for digital cytopathology evaluation and reporting include high resolution image quality, images included in interpretative reports, integration with medical records systems, appropriate mechanisms for second opinions and report addenda, and unalterable finalized reports with date and time stamps. Also, the timeline of case materials submission, evaluation, reporting, and alterations made during these processes (e.g., rescanning and/or adding new slides, reporting updates, and communications) should be documented within software systems.

High resolution imaging is crucial for accurate cytologic evaluation. Software should support high quality image capture to maintain the integrity of cellular details and include robust image processing capabilities, such as contrast enhancement and digital zoom, to improve visualization of cellular features needed for interpretation. The software should also provide tools for annotating images to facilitate communication between the clinical pathologist and clinician as well as all consulting pathologists. Annotations allow for more precise documentation of cytological findings.

Integration with practice information management systems facilitates efficient sample submission management, tracking, and electronic reporting that can be incorporated into the patient’s medical record. The software should provide secure solutions for managing digital images that are readily accessible using standard internet access. Vendors should provide robust short-term backup and archiving capabilities to ensure data integrity during case evaluation and after completion. Due to the large size of digital file formats and the expense of long-term hosting, vendor-specific archiving may not be feasible, requiring users to formulate internal policies for slide archiving and report retention that maintain compliance with governing state, provincial, or federal laws regarding medical records or regulatory requirements.

Adequate training and ongoing technical support are essential to effectively utilize software, troubleshoot issues, and maintain vendor-required software updates. Digital cytopathology vendors should offer software training programs, responsive customer support services, and have a comprehensive understanding of medical records systems that integrate with their product.

There are a few unique considerations for static image digital cytopathology. To maintain compliance with state laws regarding medical records, regulatory requirements, and/or accrediting body standards (e.g., American Association of Veterinary Laboratory Diagnosticians), all static images submitted for interpretation may need to be archived within LIMS or other record retention software, even if a cytologic description is provided. Since slide labels are not typically depicted in static images (versus WSI), pathologists should have a direct link to the images and reporting page to ensure the correct images are interpreted and reported for the correct patient.

5.3 Standard operating procedures for maintenance and malfunction

Regular cleaning and maintenance of scanning hardware and keeping associated software up to date are critical to ensuring the diagnostic accuracy of digital pathology systems. Laboratories should follow the manufacturer's instructions for maintenance. Written protocols for maintenance and troubleshooting of digital scanning equipment should be available for technicians. For recurring technical issues that arise (i.e., not an infrequent problem that can be remedied by rescanning a sample), consultation with a scanner/software technical service representative is recommended. Recalibration or a system reset may require reverification.

5.4 Accessories Required for Digital Image Review

Multiple high quality monitors are typically used for optimal sample interpretation. Suggestions for such displays include at least a 4-megapixel resolution and 27-inch diameter screen size.[15] However, this is a minimum and higher quality monitors designed for image review will improve the ability to identify smaller features, improving accurate and complete description and assessment. Monitor quality is continually improving, and specifications should be obtained from the manufacturer and the literature. The increased number and size of monitors require increased desk and workspace.

Internet upload and download speeds impact transmitting and reviewing images, pathologist TAT, and potentially image quality. Required internet speed varies based on the number and size of the image files to be transmitted, as well as other uses of the internet in the facility. Internet speed impacts cytopathology samples more than histopathology samples due to the larger file size. Facilities (clinic and reference laboratories) with onsite scanners must have high speed internet for timely upload of multiple large files, appropriate for the planned caseload. Remote pathologists must have access to reliable high speed internet so that slow download speeds do not prevent adequate specimen review. Estimated case and pathologist TAT should consider real upload and download speeds at the facilities. Currently, some companies recommend a minimum download speed of 100 megabytes per second (mbps) and upload speed of 15 mbps with a hardwired connection (not WiFi) for their digital cytopathologists. Pathologists with >200 mbps report excellent function, but again, this varies with company and exact duties of the pathologist. A written contingency plan for workflow when internet is unavailable or speeds are slowed should be in place prior to implementing a digital pathology platform. Pathologists and laboratories in remote locations should also consider the availability of an electrical generator, as loss of power may also slow workflow.

Due to the variety of hardware and software that can be used for static image interpretation, guidelines are currently lacking. However, pathologists and laboratories should ensure their standard workstations perform acceptably during validation studies (Section 6.0) before implementing static image digital cytopathology services. Clinical pathologists consulting on any electronic platform should consider the adequacy of and their comfort with their viewing platform (e.g., various computer monitors, cell phones screens, internet speeds).

6.0 Validation and Verification

Compared to glass slide cytopathology, digital cytopathology is a newly implemented method and thus requires validation before confident use for diagnostic or research purposes. The literature on digital pathology validation in veterinary medicine is currently limited. In contrast, several guidelines have been published in human medicine, reflecting the more established use of this method.[3,15,28] Goacher *et al.* reviewed 1,155 abstracts, of which 38 studies were included in the systematic analysis, predominantly focusing on histopathology cases.[43] The overall diagnostic concordance between WSI and conventional microscopy ranged from 63% to 100%, with a weighted mean of 92.4%. Kappa values ranged from 0.29 to 1.00, with a weighted mean of 0.75, indicating substantial agreement. Thus, studies suggest digital pathology is diagnostically valid, but there can be a large variation in concordance if validation and periodic verification are not performed. If the concordance is <95%, laboratories should investigate and remedy the cause or consider purchasing a different scanner or ancillary equipment.[3]

The goal of digital cytopathology validation is to ensure that digital cytopathology images are equivalent to, or non-inferior to, glass slide microscopy and that diagnosis and patient care are not compromised. The following important points are consistent in both CAP and RC Path guidelines and endorsed by this guideline:

- Every laboratory that utilizes WSI must perform validation and verification processes tailored to its specific clinical use and setting under real world conditions.
- The validation process should account for the entire system, from the scanner to the pathologist's workstation, and include any preanalytical or postanalytical components that impact quality or performance.
- Each intended system use (e.g., cytocentrifuged preparations, direct impressions, FNA biopsies, etc..) must undergo its own validation and periodic verification.
- Revalidation is required if any significant changes are made to the system (e.g., introducing a new scanner or using different displays).
- Verification is required when an instrument is replaced or a system is reset.

Validation of digital cytopathology may be approached through two complementary quality assessments:

- Image quality: Equivalence or non-inferiority of the digital image with the glass slide.
- Interpretation: Equivalence or non-inferiority of the digital cytopathology and glass slide interpretation

6.1 Digital Image Quality

Verifying that the digital scanning equipment can produce satisfactory image quality is the first validation step for digital cytopathology. Needed instrumentation varies between digital histopathology and cytopathology (Table 2).

Satisfactory quality for digital images requires:

- Representativeness (digital images targeting the glass slide areas of interest).
- Sufficient cellularity following digital scanner manufacturer guidelines.
- Adequate cell spreading, as thick preparations can impair scanning.
- In-focus images (minimized out-of-focus fields of view).
- Equivalent color and tinctorial quality to the glass slide.
- Absence of digital artifacts or distortions that could obscure cellular details.

When a scanner is considered for purchase, it is recommended that the scanner is trialed onsite in the laboratory to ensure that the scanner satisfies caseload requirements, can be integrated with the LIMS system, and can be appropriately integrated into the existing workflow. Before the onsite trial, all possible use cases should be listed in writing and an adequate number of representative samples procured for scanner assessment (not complete validation). The business case including cost of the analyzer, technologist's time, maintenance and QA versus increased revenue, improvement in workflow, access to trained personnel and pathologists can be considered before the trial and then reassessed after the trial. After a scanner is purchased, manufacturer claims should be verified and the digital workflow can then be validated.

Cytologic interpretations of digital versus glass slide cases can only be compared once the digital scanning process has been optimized and satisfactory digital cytopathology image quality is achieved. WSI is more likely to provide equivalence to the glass slide, whereas non-WSI (e.g., ROI, static images) only capture small regions of the specimen, which may result in a lack of equivalence to the glass slide. Resolution, degree of magnification, and representativeness of the sample may be limited by the quality of the camera and skill of the photographer, e.g., in the selection of areas of interest. (Section 4.0) Special stains (e.g., PAS, Prussian blue, Kinyoun's acid fast, or immunocytochemistry) represent separate use cases due to their different tinctorial properties which may not be accurately presented in digital images using settings for a standard Romanowsky stain (e.g., Wrights, Giemsa, etc.). Accurate replication of tinctorial properties varies by manufacturer and level of scanner.

6.1.1 Whole Slide Image (WSI) Sample Quality

WSI may misrepresent the sample if only some of the submitted slides are scanned, or if only a subset of a slide has been scanned. Due to the extended time required to scan cytopathology samples, submission of selected portions of samples has been recommended by scanning companies and equipment manufacturers. Additionally, many scanners may have automated systems to detect and exclude areas of suspected low cellularity or thick areas. The total scanned area must be equivalent or non-inferior to the glass slide specimen to be used in a diagnostic or research setting.

It is the responsibility of the laboratory professional validating the system and the clinical pathologists reading digital cytopathology cases to ensure that the image quality and zoom properties are adequate for accurate cytologic assessment. Rescanning or changing to true WSI instead of limited ROI should be requested if the pathologist believes the poor quality or restricted area of the scans may affect the diagnosis. (Sections 4.0 and 8.0). Potential issues include, but are not limited to:

- Out-of-focus images.
- A change in color/tinctorial properties that impacts interpretation

If the digital artifacts remain or no in-focus digital image can be obtained with rescanning, glass slide evaluation should be recommended for interpretation.

6.1.2 Non-WSI Sample Quality

The non-WSI digital cytopathology options may lack equivalence to the original glass slides if representative areas are not captured and/or the image quality is insufficient for digital cytopathology evaluation. Devices capturing non-WSI images may include WSI scanners capturing a small section of the slide (ROI) as well as devices generating static images such as microscope and cell phone cameras. The limitation related to potential non-representativeness for non-WSI settings should be disclosed in the cytopathology reports.

Training and experience in cytopathology and image acquisition enables correct targeting of representative areas with intact, well spread cells and capturing of crisp pictures. Static image acquisition, when performed by a skilled microscopist, may enable higher magnification image capture with improved resolution, allowing for evaluation of finer detail. Diagnostic laboratories and pathologists participating in the interpretation of static images should play a role in educating personnel capturing these images via continuing education seminars, videos, handouts, etc. The heterogeneity of devices, operators, number and format of images may result in greater variance of image quality.[41] Adapters that secure the cell phone camera or device to the microscope may decrease the risk of out-of-focus images. Static images and ROI that only include small areas have a higher risk of non-representativeness. Lack of operator experience in cytology increases the risks of neglecting areas of interest and/or of focusing on areas with inadequate cell preservation (lysis) or smearing (compaction), resulting in non-representativeness. (Section 4) Substandard image quality (e.g., non-representativeness, discordant color, or out of focus/poor resolution) may limit the pathologist’s ability to provide a cytologic interpretation. At the pathologist’s discretion, glass slides or digital rescan may be requested. Additionally, pathologists should provide comments about the sources of any diagnostic quality issues and suggestions for remediation. (Section 8)

Table 2. Comparison of surgical pathology and cytopathology recommendations for clinical digital pathology practice.[28]

Categories	Surgical pathology recommendation[3,44]	Cytopathology consideration
Digital pathology attribute		

Color preservation	Image and display should have color calibration	Same recommendations apply
Image compression	Image compression should not affect image quality, color integrity or introduce artifacts.	Same recommendations apply
Z-stacking (see text)		Employed to overcome 3D multiplane focus needs
Interoperability	DICOM is recommended	Same recommendations apply
Workflow and regulatory aspects		
Validation set case count (see text)	Minimum of 60 cases per use case additional 20 for new use case/ additional applications, such as IHC	60 cases per use case representing the expected spectrum of cytologic specimens and preparations. (Full revalidation if additional preparations are to be included)
Validation study design	Validation should closely emulate the real-world clinical environment for which the technology will be used. Intraobserver variability should be established with a 2-week washout period.	Similar recommendations apply
Validation composition	Cases should be representative of the expected variation in clinical cases (stains, preparation types, distribution of diagnoses)	Additional preparation and stain types beyond typical histopathology approaches are required.
Validation of components	Validation should encompass the entire workflow.	Similar recommendations apply
Competency testing	Insufficient evidence available	Consider competency needs for cytopathologists
Retention QA requirements	Documentation should be maintained recording the method, measurements, and final approval of validation for the WSI system to be used in the anatomic pathology laboratory.	Same recommendations apply. Similar metrics should be recorded on an ongoing basis for QA purposes. Ensure minimum retention requirements are met for WSI used for diagnostic purposes.

DICOM Z Digital Imaging and Communications in Medicine.

6.2 Digital Cytopathology Interpretation

Once image quality is verified, validation of digital cytopathology requires the documented proof that interpretation of the digital images is equivalent or non-inferior to the interpretation made using glass slides. This quality assessment validates that the scanner and system create digital images that have adequate slide coverage, resolution, tinctorial properties, and other quality features that allow evaluation of the samples that are not different or inferior to glass slides. In a validation study, a case may be represented by a single slide and is not required to have all slides represented digitally. The evaluator should be a board certified pathologist.[3] In validation studies for digital cytopathology processes, some cases may result in partial agreement when the digital interpretation includes some, but not all elements within the matching glass slide interpretation. Partial agreement has varying definitions in published validation documents and studies in the literature. When a validation study is being planned, it is important to clearly define full agreement, partial agreement, and disagreement criteria in the written study plan prior to data collection. The reference list may be consulted for examples in existing published studies. If the concordance is <95%, laboratories should investigate and remedy the cause or consider purchasing a different scanner or ancillary equipment.[3]

The 2022 CAP guideline revision[3] for validating WSI for diagnostic purposes evaluating surgical biopsies were used in validating telecytopathology systems and are relevant.[45,46] The authors of this guideline for validation of cytopathologic interpretation support the 3 recommendations and 9 good practice statements as follows:

Recommendations:

1. "**The validation process should include a sample set of at least 60 cases […] and reflect the spectrum and complexity of specimen types and diagnoses likely to be encountered during routine practice**."
Personnel are encouraged to use at least 60 cases/sample sets that are representative of those seen within the organization, with varying degrees of diagnostic complexity. Over-representation of frequent samples (cutaneous or subcutaneous mass, external lymph nodes, etc.) or of frequent lesions (lipoma, mast cell tumor, etc.) in the validation set could compromise the representativeness and artifactually increase the concordance.

2. "**The validation study should establish diagnostic concordance between digital and glass slides for the same observer (intraobserver variability). If the concordance is less than 95%, laboratories should investigate and attempt to remedy the cause." Additionally, "discrepant diagnoses between modalities can be subclassified as major (high risk) or minor (low risk), where major discrepancies are defined as those that would impact patient management**."
These kinds of assessments need to be made on a case-by-case basis using the question, "Would the discrepancies impact patient management?"

"**Intraobserver concordance addresses the central question of whether the same pathologist makes the same interpretation of a given case regardless of whether it is reviewed by WSI or as glass slides. The process is not intended to assess diagnostic correctness or to validate an individual pathologist's diagnostic competency**."

3. "**A washout period of at least 2 weeks should occur between viewing digital and glass slides. This recommendation is intended to address the issue of recall bias when cases are reviewed by 2 different modalities by the same observer**."
The published literature suggests a minimum of two weeks will mitigate recall bias, but longer periods may be needed.

Good Practice Statements:

1. All pathology laboratories implementing WSI technology for clinical diagnostic purposes should conduct their own validation studies.
2. Validation should be appropriate for and applicable to the intended clinical use and clinical setting in which WSI or static images will be used. Validation of WSI systems should involve specimen preparation types relevant to intended use. If a new application for WSI is contemplated, and it differs materially from the previously validated use, a separate validation for the new application should be performed.
3. The validation study should closely emulate the real world clinical environment in which the technology will be used, e.g., a laboratory system using a different scanner for reference laboratories vs in-clinic scanners provided to clients would be two different use cases with different real world environments.
4. The validation study should encompass the entire WSI system. It is not necessary to separately validate each individual component (e.g., computer hardware, monitor, network, scanner) of the system or the individual steps of the digital imaging process.
5. Laboratories should have procedures in place to address changes to the WSI system that could impact clinical results.
6. Pathologists adequately trained to use the digital system must be involved in the validation process.
7. The validation process should confirm that all of the material present on a glass slide to be scanned is included in the digital image.
8. Documentation should be maintained, recording the method, measurements, and final approval of validation for the digital system used by the laboratory.
9. Pathologists should review digital cases and glass slides in a validation set in a random order.

<u>Example</u>:

Intraobserver variation between light microscopy and digital microscopy at two time points with a minimum two-week washout was assessed in the non-inferiority design by Philips in the original FDA approval study.[20] Identical clinical information was provided to reading pathologists for both modalities. Information regarding prior diagnoses on the same patient was not provided. Reading pathologists were not allowed to request recuts or any additional special stains beyond those already provided, nor allowed to consult with other pathologists. A panel of 3 blinded adjudicating pathologists determined concordance by comparing paired diagnoses to the original sign-out diagnosis on record. Agreement was assessed as: concordant, major discordance, and minor discordance. “In keeping with widely accepted definitions, a major discordance was defined as a difference in diagnosis that would be associated with a difference in patient management.” This study provides additional detail on acceptable study design to assess concordance which may be useful for those beginning a concordance study.

6.3 Validation Report Distribution

Validation reports for digital scanners and related instrumentation should be promptly shared with pathologists enabling them to incorporate validation and quality data, supporting informed decision making. Full validation documentation must be accessible to all laboratory personnel involved in the digital cytopathology workflow. Validation certificates and summaries should be available to clinicians. Currently, there is a lack of digital validation studies and business cases in the veterinary literature and peer reviewed publication of validation and performance data is encouraged.

7.0 Training Pathologists to Read Digital Cytopathology Samples

At least an introductory level of comfort and expertise in interpreting various glass slide specimens is recommended as the foundation for subsequent training in digital image interpretation. Digital microscopy pathologists should be proficient in the quality assurance concepts of producing digital images (Sections 4.6, Table 1). Briefly, they should be able to discuss the basic principles of digital scanning, recognize common artifacts associated with scanned images, and apply basic problem-solving skills for problematic images. Static images (photomicrographs), images from scanned slides (ROI), and WSI have similar quality issues as those encountered with glass slides, in addition to the artifacts unique to the digital platform and capabilities/limitations of the particular digital system being used. Digital systems being used for training should be validated according to the recommendations in this document.

The following training recommendations are intended to be sequential and cumulative. Readers are referred to Cross et al, 2018, Appendices A through D, for further information and a case example of a training validation protocol.[15] Documentation of proficiency prior to progression to the next training stage is recommended. The timing of progression will depend on factors such as available time for training, the pathologist's competency, and the caseload's breadth and depth. Training should not be rushed and should include access to an experienced digital microscopy pathologist capable of providing feedback, discussion, and consultation.

The training recommendations in this document focus on attaining the skills needed to use digital images in diagnostic and research settings. They are not intended to replace existing expectations for cytology residency training. Organizations that wish to pursue fully remote and fully digital training are referred to Sections 5, 6, and 8 in this document for validation and quality issues that impact the calculation of diagnostic concordance. If digital training cases exist that do not have the glass slide available to the trainee, all quality issues must be articulated in training materials. Digital training cases with glass slides available are preferred to develop digital microscopy competence.

7.1 Initial Orientation for Training

Initial training in digital microscopy should include:

1. Introduction to the general principles underlying digital image capture with photography and digital image production using scanning systems.
2. Overview of the general maintenance and operation of photographic and scanning equipment.
3. Knowledge of common problems and how these appear and are detected (such as oil on the lens, problems with scanned image stitching, overly thick preparations, incomplete scans, etc.) (Section 8).
4. Detection of deficits in quality and appearance with the specific digital system being used and actions to be taken to resolve quality issues or improve any quality deficits

(Section 8).
5. Introduction to digital cytopathology images and principles of interpretation, with examples of digital images using the training organization's imaging system.

Reporting of organizational live cases (cases reported to clients) is not recommended at this stage of training. Rather, the trainee should learn how to access and use the organizational system(s), navigate around static or scanned images, and access owner, patient, and practice information within the software UI. This ensures that the cytopathology case is traceable to the submitter, correctly identified, reported for the correct preparation, and sent to the correct practice. Practice cases should be available to help the trainee learn to use the system(s).
By the end of this stage, a digital microscopy trainee should be able to:

1. Access digital images, including information about the owner, patient, history, site, specimen type, and practice affiliated with the image.
2. Understand how a high quality scanned image is produced using the organization's scanner system.
3. Navigate around the scanned image at various magnifications.

The trainee is expected to review digital images and glass slides in a training set representative of the cases and complexity encountered in the organization's daily caseload. Current American and international recommendations are to review a minimum of 60 cases with digital and glass side-by-side.[3,47] If five to six cases are assessed per day, 60 cases should be covered within two weeks, which is a minimum time devoted to study, reflection, and confidence building in the new skill of digital cytopathology interpretation and reporting. More cases may be needed and added at the discretion of the training organization.

The selection of cases by organizations should be an intentional process that may be refined over time. The training set should comprise cases representative of the species, sites, and systems the organization evaluates. Additionally, these cases should include routine and complex cases, various artifactual changes, and a variety of staining methods to present a representative spectrum of interpretive challenges.

An important goal is for individuals to determine if the digital preparation is equivalent to the glass slide preparation for interpretation. The trainee should reach the same interpretive conclusions from both digital and glass preparations while becoming familiar with the subtle differences in color, contrast, resolution, brightness, and focusing ability inherent between the two modalities and appreciating that any differences for digital images will depend on the operating system used. Blood smear reviews can be helpful at this step, since there is less variation in cell type and sample thickness for hematology slides compared to cytopathology slides. Thus, fewer variables are considered when first appreciating the differences between a glass slide and its digital image. Ideally, the decision to move to the next stage of training should be determined by agreement of the trainee and supervisor. The local training environment may provide more specific protocols for progression. At a minimum, by the end of this stage a digital microscopy trainee should be able to:

1. Consistently interpret paired digital and light microscopy training cases.
2. Evaluate paired digital and light microscopy training case specimen quality as they relate to diagnostic interpretations/conclusions.

3. Communicate the degree of certainty/uncertainty associated with the digital interpretation due to sample quality limitations either due to digital scanning or the sample itself (Section 8).

7.2 Organizational Case Reporting with Intensive Feedback

During this stage, the trainee should learn how to interpret a proportionally small caseload of digitally scanned cases and compare these to glass slides cases. The trainee is not releasing cases to the client independently during this stage. Hands-on scanning of the glass slides to create digital images may enable the trainee to recognize and troubleshoot scanning errors or artifacts better. Ideally, the decision to move to the next stage of training should be determined by agreement of the trainee and supervisor. The local training environment may provide more specific protocols for progression. At a minimum, by the end of this stage a digital microscopy trainee should be able to:

1. Provide high quality reports of digital cases suitable for clients.
2. Identify digital cases for which analysis of glass slides is indicated.

7.3 Transitional Phase with Supervisor Support

During this stage, the trainee will report digital cases as part of the organization's routine caseload. The supervisor relationship is considered more on-demand, and the responsibility for initiating supervisor support is shifted to the trainee. Ideally, the decision to move to the next stage of training should be determined by agreement of the trainee and supervisor. The local training environment may provide more specific protocols for progression. At the end of this stage, a digital microscopy trainee should be able to:

1. Provide high quality reports of digital cases for clients with an increasing caseload.
2. Identify digital cases for which analysis of glass slides is indicated.
3. Identify digital cases for which additional feedback or support by colleagues or a supervisor is desirable.

7.4 Competency Assessment in Digital Imaging Use and Interpretation

A digital cytopathology competency assessment that includes commonly encountered samples is undertaken before the digital microscopy training is completed. This should include at least 20 digital cases of various types and species representative of the organization's routine and challenging caseload.[47] However, the exact number and representative case details should be discussed by leadership and pathologists in the facility and tailored to the facility's caseload and needs. One or more supervisors review the trainee's completed assessment cases according to the facility's SOP for digital cytopathology. The assessment should be specific for digital competency, with predetermined criteria set by the organization for digital reports. Criteria for satisfactory performance on the assessment should be competency-focused and tailored to the organization, such that the trainee must integrate knowledge, skill, values, and attitudes necessary for accessing, evaluating, and reporting digital cytopathology to be successful. A high level of achievement is expected to pass the competency assessment successfully. The cytopathologist should continue to seek continuing education and support to maintain competency in digital cytopathology.

At the end of this stage, a digital cytopathology trainee should be able to assume responsibility for a full caseload, whether entirely or partly digital, as determined by the organizational standards. The individual who completes training is expected to take responsibility for seeking feedback, consultation, and collegial support as needed.

8.0 Quality Assurance Measures for Digital Case Evaluation and Reporting (Analytical and Postanalytical)

Cytopathologists should ensure that the quality of their diagnosis with digital pathology is equivalent to the current standard, conventional light microscopy. Once a cytopathologist has been trained in digital reading (Section 7), ensuring accuracy in digital reporting requires competency in identifying the slide quality factors that hinder the reading of any cytopathology sample versus quality factors that hinder image evaluation and case assessment, which are specific to the digital modality. (Table 3) Cases negatively affected by general cytopathology sample quality problems (for which diagnostic quality would not improve by glass examination) are reported out similarly when viewed digitally as non-diagnostic or inconclusive.

Table 3. Glass Slide vs. Digital Image Quality Factors Resulting in Non-diagnostic or Inconclusive Digital Cytopathology Reports

General slide quality factors (impacting glass and digital cases)	**Digital image quality factors**
- Insufficient cellularity - Cellular lysis - Necrotic material - Excess background debris that obscures cellular detail (e.g., gel material, stain precipitate) - Sample too thick/clotted - Formalin fume exposure	- Incomplete slide area scanned (WSI) - Lack of adequate focus - Insufficient fine focus for critically diagnostic details (e.g., granules, microorganisms, chromatin pattern) - Brightness/sharpness/contrast issues not immediately correctable by the platform's available real-time adjustments

8.1 Digital Case Handling Options

For problems that are unique to the digital modality, the cytopathologist can decide to manage the case in one of three ways:

1. A complete, final report is issued with no rescanning of the digital images, even though the scanned images have a flaw(s), because the cytopathologist is confident that there is sufficient visible diagnostic material and that seeing more of the sample would not

change a final report. This option is typically reserved for high quality samples with limited portions of the sample areas that are unreadable due to digital quality issues.

2. A medically useful preliminary report is issued, with the final report or an addendum completed after rescans and re-examination of new digital images. A final report could also be issued after a limited glass slide review that answers a pointed question such as, “Are bacteria present on slide 3?” This type of review is only done in instances where a digital rescan is not likely to answer the question, and it may be performed by the same or by another pathologist onsite where the slides are located. For the latter, communication of requested information and any relevant images may be placed in the LIMS for the initial digital pathologist to use in the final report. Both forms of 2-step reporting are appropriate when there is confidence that the final interpretation will not significantly differ from the preliminary one. The impact of this option on turnaround time (TAT) should be carefully considered. TAT will depend on the laboratory’s technical ability to perform any necessary rescans, such as restaining, rescanning unclear areas, expanding the scanned area of the slide(s), and/or preparing and scanning additional smears. In the event of a limited glass review, TAT may be delayed by shipment of slides to the reading pathologist if a 2nd pathologist is not onsite to process the request same-day. Laboratories may adjust TAT expectations specifically to address scenarios involving preliminary versus final or addended hematology and cytopathology reports.

3. The case is deemed not reportable via digital examination (no preliminary report is released) and is sent for evaluation using glass microscopy, either to stay within the submission TAT (i.e. too much rescanning would be needed to make the case viable digitally), or because a critical diagnostic element is beyond the current image production limits of the digital platform (e.g., suspicion for a microorganism or other diagnostic relevant item such as cytoplasmic granules).

8.2 Preliminary Reports

When deciding whether to issue a preliminary report with a subsequent addendum or a final report, the cytopathologist should consider the likelihood of a final report posing a major contradiction to the preliminary report and the potential for harm caused by initiation of treatment based on the preliminary report. For example, a final report of inflammation provided after a preliminary report that is more strongly weighted toward neoplasia would alter the perception of prognosis and require reformulation of therapeutic approaches. A final cytopathology report that significantly contradicts a preliminary report poses an unacceptable risk to patient welfare based on the conservative assumption that pathologists are usually not in direct contact with submitting clinicians, and that preliminary interpretations may lead to interventions before receipt of the final report. It is acknowledged there would be less inherent risk in scenarios where pathologists and clinicians are in close communication (e.g., same building, pre-report consultation by phone, etc.).

Preliminary digital cytopathology reports should:

- Be transparent about the reason for rescans and reexamination. For example, a slide was rescanned because a significant region of the initial scan was out-of-focus.

- Have a date and time stamped addendum attached as an official record of full case evaluation once rescans with re-examination by the pathologist are complete, even if the addendum states that there are no additional findings.

8.2.1 Small Microorganisms and Other Fine Detail

Small microorganisms (e.g., bacteria) and other subcellular details may be beyond the current limits of the scanner or difficult to visualize based on the digital platform or scanner's resolution limitations, such that rescanning would not be beneficial. Both the diagnostic and clinical contexts of the suspected differentials are important for deciding whether to proceed with digital or glass interpretation in cases suspicious for bacteria or abnormal subcellular structures of similar size. For example, sensitivity for diagnosis of *Mycoplasma* spp. is low for both glass and digital blood smear evaluations.[48] Therefore, when the case history and CBC results support a possible Mycoplasma infection, recommending PCR during a pathology review of a digital blood smear, without an intervening glass slide evaluation, does not compromise quality and maximizes TAT. Scanning digital blood film or digital cytopathology images solely for Mycoplasma sp. identification or evaluation of similarly sized structures is not recommended using existing technology.

Another example involves the location and severity of infection. For example, consider the different clinical consequences of a false negative for bacteria in an acral lick granuloma where infection is assumed to be present versus a false negative for pyothorax. If the cytopathologist is suspicious that bacteria are present in the digital evaluation but cannot completely confirm due to lack of sufficient resolution, there are two possible actions which may be appropriate based on the pathologist's judgement: 1. Request glass slide evaluation (8.1 reporting option 2 or 3 above). 2. Report impression from digital images with recommendations for follow up testing.

8.3 Incomplete Scans and Out-of-Focus Scans

Upon opening a case, the first step is evaluating how much of the total slide area can be read (scanned and in-focus) in conjunction with sample cellularity. As a cytopathologist evaluates a digital case using WSI, it is important that the digital platform should allow viewing of a small thumbnail image of the entire slide. The scanned area is typically outlined within the thumbnail and may be the entire slide or a subset, as most platforms allow both automated and manual options for selecting the scanned area(s) (Section 4.4). The pathologist can then determine what fraction of each slide has been scanned and whether rescanning will be needed.

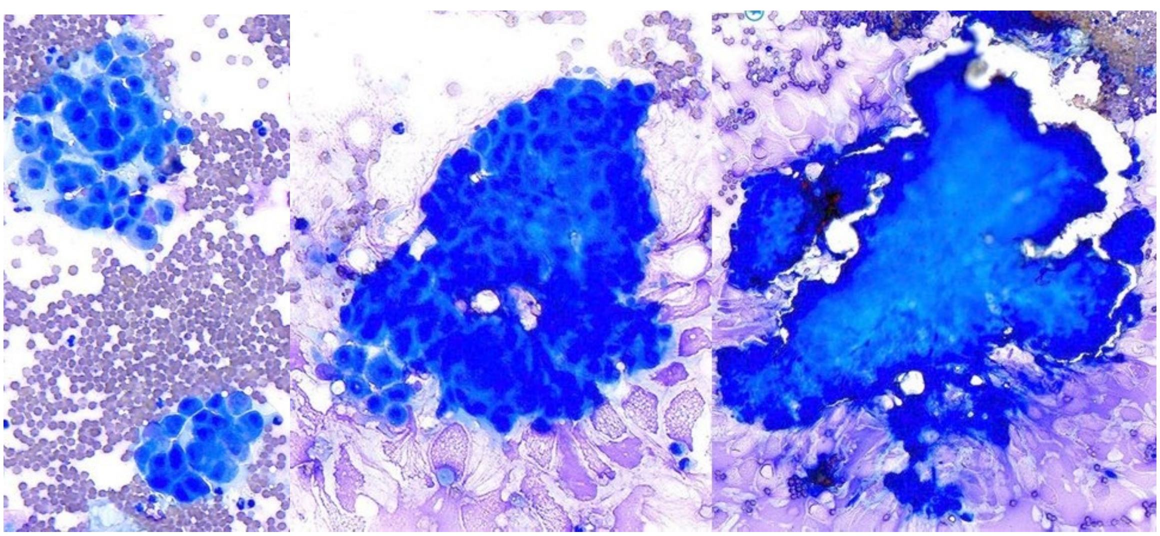
8.3.1 High Cellularity Samples

The more cellular the sample, the higher the tolerance for reporting when a portion of the sample is unreadable. For example, if three of four total slides are in-focus with abundant mast cells, the pathologist may feel more comfortable that the sample is representative than if mast cells were sparse on the readable areas. A guiding principle can be, "What is the likelihood that another pathologic process is present on the part(s) of the slide(s) that is not readable?" If there is confidence for a specific diagnosis but concern that the readable portion of the slides may not be representative, this is a scenario in which a preliminary report of likely mast cell neoplasia may be released with a comment that the incompletely scanned or out-of-focus area(s) will be rescanned and reread with an addendum to follow.

There is also a subset of situations for which some out-of-focus material may be interpreted as the same as in-focus material found within the same case slides with similar microscopic patterns, because the focus is only mildly compromised (compare Figures 1A and 1B). This is similar to interpretation of a thick preparation on a glass slide. For example, tight clustering of epithelial cells (if there is no confounding factor, e.g., other cell types are present) which can still be visualized without complete individual cellular detail (Figure 1C). Similarly, good digital focus at the edges of thick areas can often be interpreted when central regions are out-of-focus. Professional judgment is exercised in determining how representative the readable cells are. The pathologist should refrain from speculating when image quality is too compromised for confident interpretation. Glass evaluation may improve depth of focus and be an option but may not improve patient care or diagnosis if it does not improve the diagnostic quality of a thick preparation.

Figure 1: Three microscopic images from the same cytology slide of prostate tissue obtained by fine needle aspiration, from good (A) to poor (C) resolution. In the absence of other pertinent parenchymal populations, it is reasonably inferred that the cells in images B and C are of similar epithelial nature as those clearly identified in image A, and per the pathologist's discretion, a preliminary report may be released. A: Wright Giemsa, 20x obj; B,C: Wright Giemsa, 40x.

A] B] C]



8.3.2 Low Cellularity Samples

Low cellularity samples should have a stricter threshold for completely scanned and in focus slide area when issuing a report, due to more questionable representativeness. Digital view settings, such as gamma, should be used to enhance the visibility of low numbers of cells and biologic material. The thumbnail image can be particularly useful even though it is small, as it is often easy to see stained areas of abundant biologic material that may not have been scanned (Figure 2). Conversely, some low cellularity samples do not have visible areas of biologic material on the thumbnail (Figure 3). Because it is unknown how much cellular material is actually present in these unscanned areas, preliminary reporting or sending for glass reading should be chosen to ensure that all available biological material has been examined.

Figure 2: Thumbnail image of a cytology slide with the scanned area outlined by a green box. If no or rare cells are seen in the scanned images and there is a clearly visible area of unscanned biologic material on the thumbnail, requesting evaluation of the glass slides or issuing a preliminary report with subsequent rescans is warranted. Wright Giemsa, Motic scanner thumbnail (< 2x objective magnification)

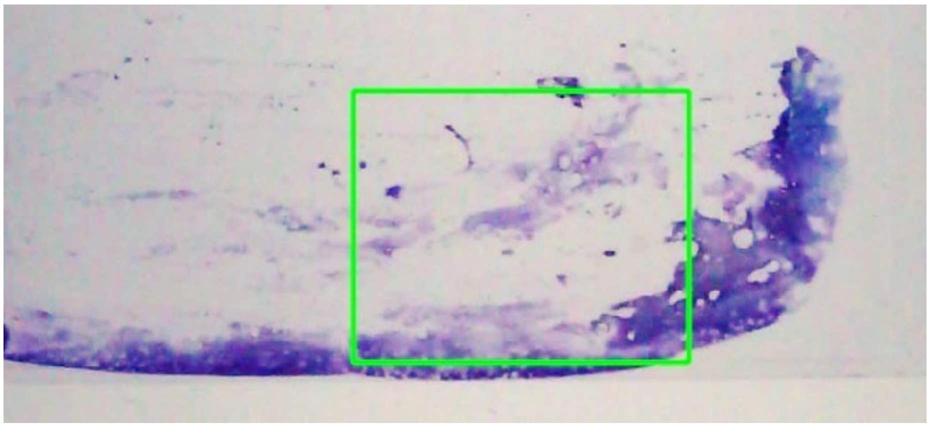
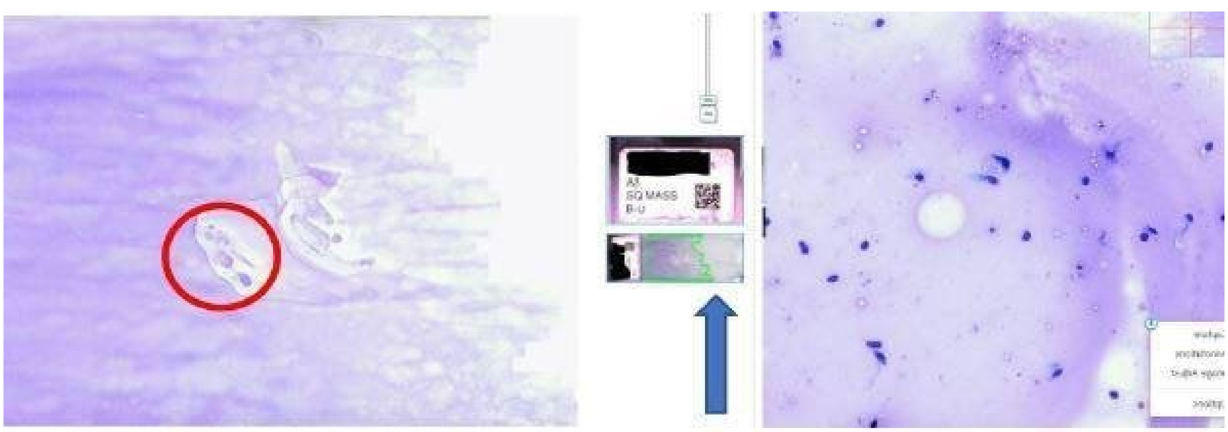


Figure 3: The right side of image [A] shows a thumbnail image of an entire cytology slide with a missing area in the scan (blue arrow; scanned area is outlined in green) [B] Higher magnification of the red circled area of [A] shows few mesenchymal cells that are not visible on the small thumbnail nor at lower magnification. Although the unscanned area does not look cellular, this case is a candidate for preliminary reporting or complete glass reading if the pathologist suspects that there may be material in the unscanned area that might contradict a preliminary report. Wright Giemsa. A: 4x objective; B: 20x objective.



[A] [B]

In conclusion, when evaluating a case's initial scanned images (if incompletely scanned or focused), guiding questions include:

1. Does the available sample adequately support the conclusion(s)?
2. Is there a reasonable possibility that seeing more of the sample may change the interpretation?
3. What limitations or potential for additional conclusions should be stated as a result of

sample and digital image quality concerns?

If most of the sample can be seen (small out-of-focus or missing areas) and there is a confident interpretation, then seeing the complete slide will not likely add enough value to prevent issuing at least a preliminary report. It is also important to note, especially for low cellularity samples (and similar to traditional glass reading), whether what is seen is characteristic for the patient signalment, anatomic location, reported chronicity, and/or gross description. If not (such as only rare adipocytes in a sample described as from an ulcerated cutaneous mass), this would give more cause for either digital rescanning or requesting glass slide in a case that has incomplete digital readability. If available to the pathologist, colleague consultation for a second opinion on the reporting choice when there is some digital image quality compromise is beneficial.

Similar approaches can be utilized for static images, where it is known that portions of the slides are not depicted. Causes of inadequate static images of an otherwise diagnostic whole slide include non-representative images, insufficient numbers of images, poor microscope settings, suboptimal camera settings, and post-capture over-editing. Ideally, diagnostic services should have options for clients to submit glass slides or fluid samples for full evaluation. Pathologists should utilize their knowledge of potentially unrepresentative samples when drafting reports.

Static image findings must be correlated with the anatomic location, lesion description, clinical history, signalment, and the overall image quality (Section 4). Reports should clearly identify that static images were evaluated (versus glass slide or WSI) and should contain statements presenting the limitations of static image evaluation or any assumptions made during static image interpretation. Inserting captured images from the scanned slides or the submitted static images in the final report as photodocumentation is often beneficial when further review of the submission is performed.

9.0 Conclusion

This document reviewed the recent relevant digital pathology history and literature in human and veterinary medicine including existing international and domestic guidelines. Quality guidelines for preanalytical, analytical, and postanalytical components of digital cytopathology case presentation are provided in this document, which do not typically represent best practice, but can be used as a baseline for digital cytopathology systems and training.

Preanalytical requirements necessary to generate a digital diagnostic case equivalent to that of a glass slide are similar to those for conventional cytologic interpretation. Achieving equivalency requires attention to details associated with sampling, submission, slide processing, and additional adaptations to the UI. Possible loss of fine focus and truncated scanning area associated with WSI necessitate proper sample preparation and placement. Review of static images requires submission of sufficient numbers of images at multiple magnifications to provide an accurate depiction of the sample.

Appropriate training of operators and provision of pertinent clinical data and physical sample characteristics to pathologists can result in the desired equivalency between digital and conventional cytopathology. Therefore, all personnel involved in digital case preparation must participate in the requisite training modules which should include glass slide cytology sample preparation, detailed user instruction on the software and UI, and the digital scanner itself. (Section 4)

Successful implementation of digital cytopathology relies on carefully validating scanning hardware, software, and data storage systems. Digital pathology scanners vary in cost, optical resolution, and throughput, so the requirements must be tailored to the workflows and needs of individual laboratories. Variables such as laboratory context (e.g., reference laboratory vs. POC), caseload, sample types, IT configuration, system integrations, and operator training all play a role in determining the optimal setup. Software must ensure secure medical record integration, high-resolution image processing, and a UI that facilitates submission of adequate information for interpretation. Optimal performance of these systems requires standardized operating procedures for hardware upkeep and software updates. Finally, high resolution imaging, high quality monitors and reliable high speed internet and electricity are recommended to optimize image interpretation and timely sample review. (Section 5)

The International Organization for Standardization has defined validation as the confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled.[16] The College of American Pathologists has adopted this definition and used laboratory and scanner-specific concordance studies to provide objective evidence that there is equivalence or non-inferiority of digital images compared to glass slides. Recently, the American Society of Cytopathology (ASC) has adopted the CAP guidelines in large part with additional recommendations and considerations for cytopathology.[3,28] After image quality assessment and validation, diagnostic concordance between digital and glass slides for the same observer (i.e., intraobserver variability) must be established. If concordance is less than 95%, laboratories should investigate and attempt to remedy the cause. The ASVCP Quality Assurance and Laboratory Standards Committee aligns with these existing guidelines and endorses their recommendations for validation. (Section 6)

Training cytopathologists for digital cytopathology is recommended to occur in a scaffolded, sequential manner with feedback and supervision and conclude with a competency assessment. It should leverage an internally curated set of training cases comprised of paired digital and glass samples representing the breadth and complexity of cases evaluated by the organization. The training sequence should conclude with a competency assessment using a set of digital cytopathology cases appropriate for the organization that requires demonstration of expected knowledge, skills, values, and attitudes for live digital case reporting within organizational standards. The individual who completes training is expected to assume responsibility for seeking feedback, consultation, and collegial support as needed. (Section 7)

For samples with digital-related quality issues (e.g., incomplete scans, out-of-focus areas, poor fine focus, and poor, unadjustable contrast/sharpness/brightness), the decision must be made, based on the severity of the quality issues (Section 8), to either:

(1) report digitally on first pass, when there are minor quality issues and high confidence that further examination will not alter diagnostic conclusions.
(2) report using both a preliminary report and an addendum after improved rescans have been examined, or after a limited glass slide check has been performed. The quality-related limitations necessitating the 2nd step of processing should be mentioned in the preliminary report.
(3) send the case for complete glass reading/reporting.

A guiding principle for deciding between these reporting modes is whether seeing more of the sample would likely enhance diagnostic quality enough to change the interpretation and clinical recommendations.

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