Technical Note

Implementation of Collodion Bag Protocol to Improve Whole-slide Imaging of Scant Gynecologic Curettage Specimens

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Abstract

Background: Digital pathology has been increasingly implemented for primary surgical pathology diagnosis. In our institution, digital pathology was recently deployed in the gynecologic (GYN) pathology practice. A notable challenge encountered in the digital evaluation of GYN specimens was high rates of scanning failure of specimens with fragmented as well as scant tissue. To improve tissue detection failure rates, we implemented a novel use of the collodion bag cell block preparation method. **Materials and Methods:** In this study, we reviewed 108 endocervical curettage (ECC) specimens, representing specimens processed with and without the collodion bag cell block method ($n = 56$ without collodion bag, $n = 52$ with collodion bag). **Results:** Tissue detection failure rates were reduced from 77% (43/56) in noncollodion bag cases to 23/52 (44%) of collodion bag cases, representing a 42% reduction. The median total area of tissue detection failure per level was 0.35 mm^2 (interquartile range [IQR]: 0.14, 0.70 mm^2) for noncollodion bag cases and 0.08 mm^2 (IQR: 0.03, 0.20 mm^2) for collodion bag cases. This represents a greater than fourfold reduction in the total area of tissue detection failure per level ($P < 0.001$). In addition, there were no out-of-focus levels among collodion bag cases, compared to 6/56 (11%) of noncollodion bag cases (median total area = 4.9 mm^2). **Conclusions:** The collodion bag method significantly improved the digital image quality of fragmented/scant GYN curettage specimens, increased efficiency and accuracy of diagnostic evaluation, and enhanced identification of tissue contamination during processing. The logistical challenges and labor cost of deploying the collodion bag protocol are important considerations for feasibility assessment at an institutional level.

Keywords: Collodion bag, digital pathology, endocervical curettage, gynecologic, whole slide imaging

Introduction

Recent technological advancements in the digitization of pathology glass slides provide an unprecedented opportunity for innovations in clinical diagnostics, research, and education. The College of American Pathologists (CAP) has issued guidelines for the validation of whole-slide imaging (WSI) for diagnostic purposes,[1] and the Food and Drug Administration has approved two integrated digital pathology systems (Philips IntelliSite Pathology Solution [PIPS] and Leica Aperio AT2 DX), as well as a digital pathology software module (Sectra, Sweden) for primary diagnosis. Moreover, numerous studies have demonstrated the diagnostic noninferiority of WSI to glass slides.[2-4] Despite this apparent increase in acceptance of digital pathology as a diagnostic modality, only a few pathology laboratories in the US have transitioned to a digital practice due to numerous operational, information technology (IT), financial, and cultural barriers of adopting WSI for routine diagnostic use.

At our institution, we have systematically deployed digital pathology (PIPS) for primary diagnosis in subspecialty waves. The neuropathology service was first to validate and

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demonstrate feasibility of a fully digitized practice. Following a successful neuropathology validation, we moved on to the gynecologic (GYN) pathology practice to "stress-test" our workflow with a high-volume service. While we faced numerous operational and IT-related challenges during this transition (a manuscript reporting our experience is in preparation), in this report, we aim to focus on a notable challenge encountered by us and others[5] during the evaluation of GYN specimens: scanning failure of specimens with scant tissue. We noted a significant portion of endocervical curettage (ECC) specimens (particularly those with limited tissue quantity), demonstrating high tissue detection failure rates in the form of (1) unscanned tissue [Figure 1], (2) partially scanned, transected tissue fragments [Figure 2], and (3) out-of-focus scans [Figure 3]. Discussions with Philips representatives confirmed the possibility of tissue fragments <0.4 mm × 0.4 mm being missed by their scanning algorithm. The CAP guideline for WSI validation states, "the validation process should confirm that all of the materials present on a glass slide to be scanned is included in the digital image" (Guideline statement #11).[1] As such, we adopted a hybrid digital/glass-slide approach to the evaluation of GYN specimens, in which we recommend the use of glass slides for the evaluation of specimens with scant tissue, particularly ECC specimen.

In an attempt to mitigate tissue detection failure, we considered a novel use case of a well-established specimen preparation protocol within the practice of pathology: the collodion bag technique.[6,7] The collodion bag is traditionally used in cytology as a cell block preparation method to concentrate and enhance scant specimen capture.[8,9] Briefly, the protocol involves

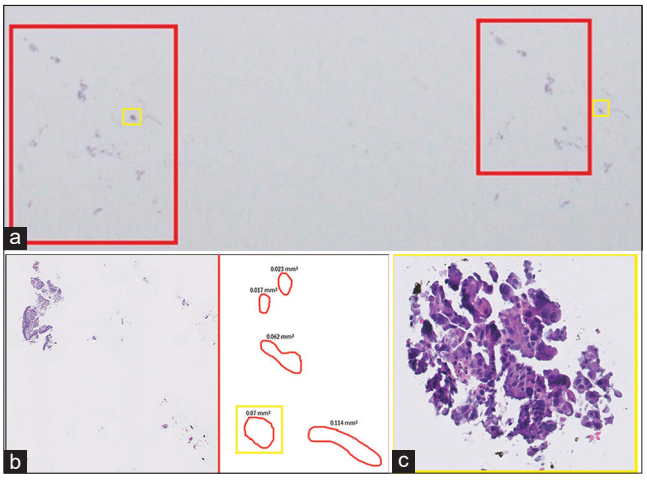

Figure 1: Example of tissue detection failure with unscanned tissue (a) Macro image of slide with two-level sections. The red boxes indicate scanned region of interest. The right level section shows tissue fragments that are outside the scanned region of interest, indicating tissue missed for high-resolution scanning. The yellow box marks a fragment of adenocarcinoma *in situ* that is missed. (b) Portion of whole-slide image of the same slide. The red vertical line divides the area scanned at high resolution (left) and area skipped for scanning (right). Fragments of missed tissue marked by red free-form line. Yellow box indicates missed adenocarcinoma *in situ*. (c) High-resolution image of missed adenocarcinoma *in situ*

pipetting cytology specimen into a conical tube lined by the collodion bag and centrifuging the suspension down to a pellet. The bag is then taken out of the tube, tied off, and submitted in a cassette for routine processing in the histopathology laboratory. The result is a round cell block section with tissue concentrated and contained within a well-visualized, pale eosinophilic rim formed by the collodion bag [Figure 4a]. Given the similarity in the scant and fragmented nature of cytology and ECC specimens, we hypothesized that the collodion bag technique will be well suited for the containment and concentration of endocervical tissue, leading to enhanced tissue detection and improved scanning efficacy. Herein, we report in detail our challenges with the scanning of scant ECC specimen and the marked improvements observed with the use of the collodion bag cell block method.

Materials and Methods

We reviewed 108 ECC collected from May 2019 to March 2020, representing specimens processed with and without the collodion bag cell block method (n = 56 without collodion bag, n = 52 with collodion bag). Specimens represented a wide distribution of endocervical diagnoses, including benign endocervix, low-grade squamous intraepithelial lesion, high-grade squamous intraepithelial lesion, adenocarcinoma *in situ*, squamous cell carcinoma, and endometrial adenocarcinoma. The study protocol was approved by the Institutional Review Board.

We initiated application of the collodion bag technique (already utilized in our cytology division) to the preparation of all ECC specimens (regardless of tissue quantity, clinical diagnosis, clinic of origin, and any other factors) upon departmental approval. The collodion bag cell block protocol follows the methods initially reported by Fahey and Bedrossian[7] and subsequently modified by Rollins and Russel[10] and

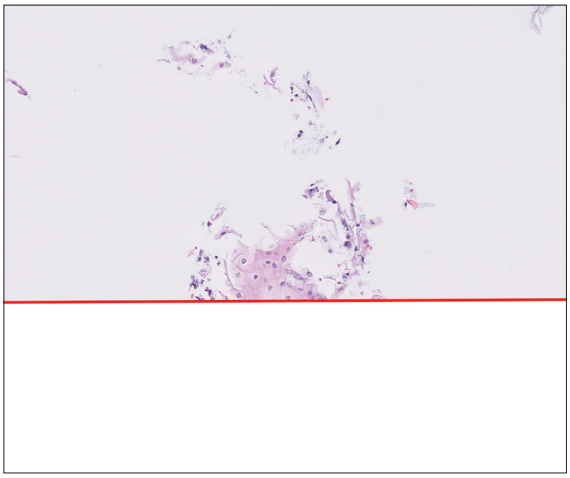

Figure 2: Example of partially scanned, transected tissue fragments (one portion of the tissue is within the scanned region of interest, but another portion of the same fragment is outside of the scanned region of interest and not scanned at high resolution). The red horizontal line divides the area scanned at high resolution (top) and areas skipped for scanning (bottom)

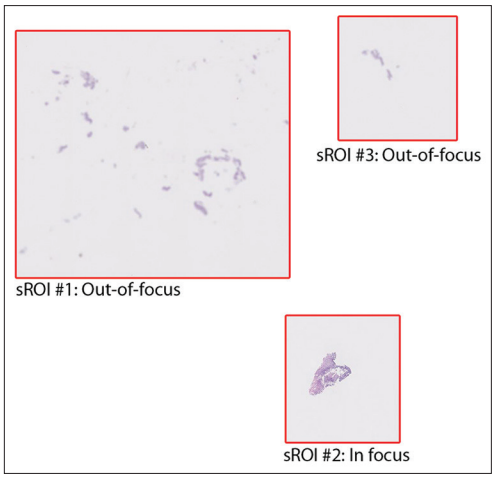

Figure 3: Example of out-of-focus scans. One-level section of this curettage specimen generated three scanned region of interests (each outlined by red boxes) due to scattered tissue distribution. SROIs #1 and 3 are out of focus, while scanned region of interest #2 is in focus

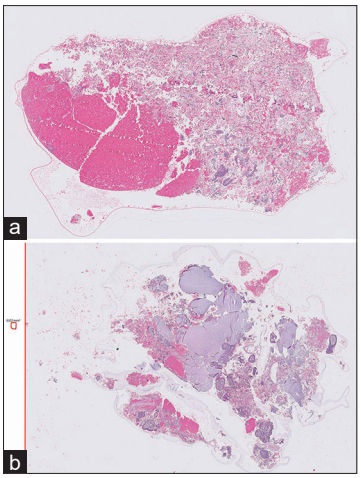

Figure 4: Sections of endocervical curettage specimens prepared with the collodion bag technique. (a) Tissue fragments are concentrated and contained within the collodion bag visualized as an eosinophilic rim surrounding the tissue. (b) Example of breakage in collodion bag that results in tissue spillage and an area of tissue detection failure. The red vertical line divides the area scanned at high resolution (right) and area skipped for scanning (left). A fragment of missed tissue is marked by the red free-form line

Wilgenbusch *et al*.[9] Our methods differ from these three studies in which we coat the test tube with collodion (Macron Fine Chemicals) through transfer of the collodion fluid between two tubes at least seven times to thicken the collodion bag coat [Table 1]. This enhances the durability of the bag, as well as its visibility on histologic sections. Once the coating is complete, we fill the tubes with normal saline, cover with parafilm, and store them upright in the refrigerator. Our cell block preparation methods differ from those used by the aforementioned studies in that we utilize the lowest rpm and centrifugation time [Table 2].

Slides were scanned and evaluated using PIPS. A macro image of a slide represents a low-resolution snap shot of the entire glass slide that is present at the top right corner (default setting) of the Philips Image Management System (IMS). As the macro image is not subject to any automated tissue detection algorithm, it is used as the standard to detect all tissues present on the glass slide. If a tissue fragment is present on the macro image but not on the scanned region of interest (sROI), the tissue was missed for high-resolution scanning. Tissue detection failure was identified in the forms of: (1) unscanned tissue (completely outside of the sROI) [Figure 1], (2) partially scanned, transected tissue fragments (one portion of the tissue is within the sROI, but another portion of the same fragment is outside of the sROI and not scanned at high resolution) [Figure 2], and (3) out-of-focus scans (the fragments are within sROI but not scanned at the correct focus depth) [Figure 3]. Each ECC specimen was evaluated to identify the level section with the largest total area of tissue detection failure for further analysis. Areas of failed tissue detection were digitally annotated and quantified in the Philips IMS (v3.2; using the free-form tool) and the total surface area was calculated. As needed, the corresponding glass slide was reviewed to determine whether these unscanned areas indeed represented missed tissue (vs. irrelevant debris) and if they contained diagnostic tissue. The tissue detection failure frequency and areas were compared between cases prepared with and without the collodion bag method using a Chi-squared test and Mann–Whitney U-test, respectively, using the statistical analysis software R, version 3.4.2 (R Core Team 2019, Vienna, Austria).[11]

Results

Tissue detection failure occurred in 77% (43/56) of noncollodion bag cases and 23/52 (44%) of collodion bag cases [Table 3]. This represents a reduction of tissue detection failure rates by 42%. While the collodion bag cell block preparation method kept majority of the specimen inside the bag, tissue spillage during histologic processing [Figure 4b] did occur. The median area of tissue detection failure per level was 0.35 mm^2 (interquartile range [IQR]: 0.14, 0.70 mm^2) for noncollodion bag cases and 0.08 mm^2 (IQR: 0.03, 0.20 mm^2) for collodion bag cases. This represents a greater than fourfold reduction in total tissue detection failure area per level ($P < 0.001$). In addition, there were no out-of-focus slides among collodion bag cases, whereas 6/56 (11%) of noncollodion bag cases had out-of-focus areas (median = 4.9 mm^2). In some noncollodion bag cases, there were areas with alternating high resolution and out-of-focus areas within the same level [Figure 3].

Tissue detection failure was readily assessed on the macro image view in a majority of cases, and we reviewed the glass slides for a subset of cases where determination of suspected tissue detection failure was challenging. The glass slide was additionally reviewed to determine if diagnostic tissue was present in unscanned tissue. Of note, the sROI varied

Table 1: Comparison of collodion bag preparation methods*

Variable	Stanford health care	Fahey and Bedrossian (1993)	Rollins and Russell (2017)	Wilgenbusch *et al*. (2020)
Collodion coating	Transfer between two tubes at least 7 times	Immersion for 10 min	Immersion for 10–15 min	Immersion for 1 h
Drying time	20–30 min	Until dry	10 min	1 h
Opacity	Discard if opaque	Subsides when dry	Discard if opaque	Discarded if also wrinkled
Storage medium	Normal saline	Dry	Distilled water	Tap water
Storage orientation	Upright	Upside down	Upright	Upright

*Modified from Wilgenbusch *et al*. 2020

Table 2: Comparison of cell block preparation methods*

Variable	Stanford health care	Fahey and Bedrossian (1993)	Rollins and Russell (2017)	Wilgenbusch *et al*. (2020)
Formalin fixation	Precollodion tube	Postcollodion tube	Postcollodion tube	Precollodion tube
Centrifugation	5 min/600 rpm	8 min/1500 rpm	10 min/2500 rpm	5 min/2700 rpm
Supernatant	Pipetted	Pipetted	Pipetted	Poured
Securing	Tied with cotton string	Folded	Tied with cotton string	Clamped
Removing excess	Cut	Cut	Cut	Torn

*Modified from Wilgenbusch *et al.* (2020)

Table 3: Comparison of frequency and area of tissue detection failure between cases prepared with and without the collodion bag method

		No Collodion Bag ($n=56$)	Collodion Bag ($n=52$)	*P*
Missed	Frequency	43/56 (76.8%)	23/52 (44.2%)	<0.001
	Median area (25, 75%)*	0.35 (0.14, 0.70)	0.08 (0.03, 0.20)	<0.001
	Maximum area*	11.8	1.2	
Out-of-focus	Frequency	6/56 (11%)	0/52 (0%)	
	Median area (minimum, maximum)*	4.9 (0.3, 30.4)		
	*mm^2			

from level to level within the same specimen. Therefore, tissue missed for scanning on one level may be caught on a subsequent level. Undetected diagnostic tissue fragments included adenocarcinoma *in situ* [Figure 1] and high-grade squamous intraepithelial lesions. However, these diagnostic tissue fragments were present within the sROI on other levels on the same slide.

Discussion

A fundamental attribute critical to the use of WSI for primary diagnosis is that scanned slides are completely accurate reproductions of glass slides. However, given that scanners may employ automated algorithms that detect tissue to determine areas to be scanned (or skipped if tissue is presumed to be absent), they are susceptible to errors that may miss small fragments of tissue. Missed diagnostic tissue is a major safety issue that may have devastating consequences for the patient, the pathologist, and the viability of WSI as a diagnostic medium. The CAP guideline for WSI validation recommends confirmation of all materials present on the glass slide to be included in the digital image (Statement #11);[1] however, the guideline does not propose a practical solution. A recent report by Fraggetta *et al*. studied various types of scanning errors, including missed tissue due to automated tissue finder failure, and recommended the use of macro images as a quality control (QC) measure to detect missed tissue.[12]

In our endeavor to validate WSI for GYN pathology, we found that scant curettage specimens predictably experience scanning failure at a high rate. Prior to the implementation of the collodion bag technique, the majority (77%) of digital scans of ECC specimen slides had tissue detection failure in at least one level section. This figure is nearly identical to those reported by Rabban *et al*. (75% detection failure rate in ECC specimens).[5] While lesional tissue was missed in two noncollodion bag cases, these fragments were caught on other level sections with larger sROI. A significant improvement in tissue detection was observed after the implementation of the collodion bag protocol, with a >40% reduction in tissue detection failure rate. When tissue detection failure occurred in collodion bag cases, this was mostly attributable to tears in the collodion bag caused by histologic processing that releases small tissue fragments susceptible to scanning misses [Figure 4b]. Regardless, the collodion bag significantly limits tissue dissipation, leading to a greater than fourfold reduction in the total amount of missed tissue (area, mm^2). In addition, the concentration of tissue, as well as the distinct eosinophilic rim formed by the collodion bag, appears to greatly help the scanning algorithm in picking

the appropriate scanning depth, as there were no out-of-focus scanned images of the collodion bag cases. No lesional tissue was missed in the examined collodion bag cases; however, this possibility is not excluded given that tissue can be missed even with collodion bag preparation.

Additional benefits of the collodion bag included ease of slide evaluation due to tissue aggregation in a central area of the slide. Without a collodion bag, ECC tissue tends to scatter throughout the slide and can be very time consuming to evaluate. The perceived increase in evaluation efficiency of collodion bag cases was universal among GYN pathologists with significantly decreased concern for missed lesional tissue. Furthermore, the collodion bag effectively excluded tissue "floaters" such that contaminants from other specimens were kept outside of the bag and thus readily identified [Figure 5]. Both of these benefits, while not directly related to scanning efficacy, are significant tissue preparation improvements that lead to workflow efficiency and improved patient care.

While we observed a marked improvement in tissue scanning with the use of collodion bag, the method does not completely prevent missed tissue. Therefore, even when using collodion bag, we advise pathologists to exercise caution for missed tissue with a low threshold to convert to glass slide evaluation. As recommended by Fraggetta *et al*.,[12] evaluating macro images for unscanned tissue fragments can be very effective. In addition, we look for transected tissue at the edge of sROI as indication that tissue is being missed. In our hands, out-of-focus scans appear to be largely prevented by collodion bag preparation. One may argue that once lesional tissue is identified on a scanned slide, it is unnecessary to review the glass slide even if there is suspected unscanned tissue. While this practice may be acceptable if the discovery of additional lesional tissue leads to no difference in clinical management, it is our opinion that subspecialty expertise, as well as familiarity with institution-specific management protocols, is necessary to make this decision, and such an approach must be taken with significant caution.

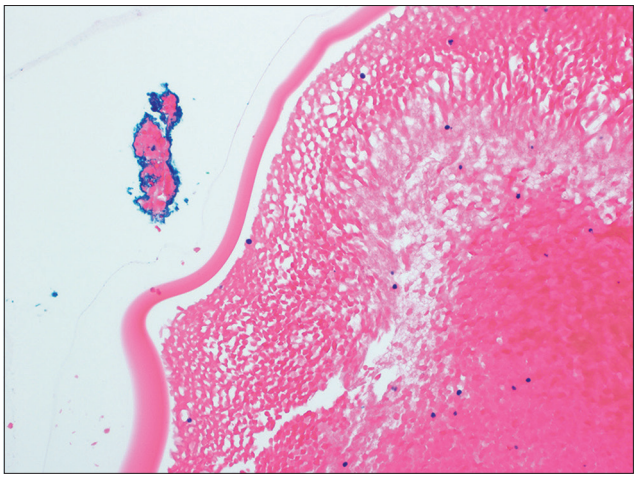

Figure 5: Illustration of tissue floater (inked blue) from a separate specimen identified outside of a collodion bag cell block

A limitation of our study is that we only evaluated the collodion bag protocol for ECC specimen. However, given how well the collodion bag protocol works on a variety of cytology specimens as well as ECC specimens, we have no reason to believe that its performance will be any different for other scant/fragmented surgical specimens. The reason for limiting our initial evaluation to ECCs was largely due to operational practicality. The challenges of large-scale implementation of the collodion bag method include its labor-intensive nature and integration into existing workflows. The time to prepare the collodion bag is approximately 10 min and an additional 45 min for the cell block. There is also a 15-min drying step and if the collodion bag is made for later use, it is a 10-min storage preparation step. The material cost for the collodion is $650 per 1000 bags ($0.65 per specimen) without accounting for labor cost. The material costs are minimal compared to the labor costs associated due to the manual nature of the protocol in its current form. We considered using commercially available collodion bags, but these were physically too large and impractical for our application. A collaborative adjustment to the surgical pathology and cytopathology workflow was also needed since colposcopic specimens are traditionally handled by pathology assistants in surgical pathology, but collodion bag production and cell block preparation are handled by cytology prep-techs. Additional training was also needed for histotechnologists since the collodion bag string needs to be removed prior to cutting paraffin-embedded blocks, and the outcome of collodion bag sections are noted to be particularly operator dependent.

Digitization of a glass slide is a complex process that requires flexibility and creative problem-solving for workflow optimization. This is a shared responsibility among pathologists, technical staff, and scanner manufacturers. To address the issue of missed tissue fragments, we have implemented the collodion bag technique which has resulted in significant scanning improvement. We also continue to be vigilant about the possibility of missed tissue and maintain a low threshold to convert to glass slide evaluation. The possibility of a more rigorous quality check of images upfront, including manual override of the sROI, was considered; however, this type of manual QC is suboptimal in the setting of high case volume because digital pathology, in the long run, should promote efficiency and automation. It is our hope that manufacturers will continue to innovate and advance their technology to reduce missed tissue. Deployment of WSI for clinical care is a work in progress, and it is important to understand strengths and limitations of the digital pathology platform before adopting a 100% digital diagnostic environment.

Conclusions

We demonstrated that the collodion bag cell block preparation method significantly improved the digital image quality of fragmented and scant GYN specimens. There are also benefits independent of digital pathology, including efficiency and accuracy of diagnostic evaluation and identification of tissue

contamination. We expect that collodion bags can be applied broadly to other areas of surgical pathology where scant and fragmented tissue specimens are common. Incorporating this method requires integration of a new workflow and appropriate staff training to make a labor-intensive process possible to achieve and scale-up.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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