



# Utility and Practicality of Multi-level Sectioning and Upfront Unstained Slide Cutting in Head and Neck Biopsies: A Critical Analysis

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## Abstract

Upfront interval sectioning (cutting unstained slides between H&E levels) is used at our institution for biopsies at all sites except the gastrointestinal tract. Very limited data exists in the literature for the need for interval sectioning, and we are aware of no data at all for the head and neck. Biopsies from the larynx, oral cavity, pharynx, and sinonasal tract at our institution have had 5 levels cut. Levels 1, 3, and 5 or levels 2 and 5 had been stained with hematoxylin and eosin (H&E), depending on the subsite, and the remaining slides saved for possible later use. We retrospectively evaluated the use of unstained slides at these sites for clinical utility and efficiency by analyzing 3 years of cases from 1/1/2014 to 12/30/2016. A cutoff of 10% utilization was considered justification for continued upfront unstained slide cutting. We collected 706 larynx, 572 oral cavity, 184 pharynx, and 85 sinonasal tract biopsies over 3 years. The overall rate of unstained slide usage was 18.2%. Usage rates were significantly different by site: 7.8% (55/706) for larynx, 21.9% (125/572) for oral cavity, 30.6% (26/85) for sinonasal tract and 40.8% (75/184) for pharynx ( $p < 0.0001$ ). The most common stain ordered in the pharynx was p16 immunohistochemistry (59.7%), but it was Grocott methenamine silver staining in the larynx (74.5%), oral cavity (70.4%), and sinonasal tract (35.1%). Usage of unstained slides was lowest for the larynx, and review of the biopsies with unstained slides utilized showed that the lesion was present on the 3rd H&E level in all cases. Removing this practice would have translated to saving 1,378 unstained slides. Upfront interval sectioning makes practical sense for biopsies from most sites in the head and neck, especially the pharynx, but our data suggests it can reasonably be forgone at least for biopsies of the larynx.

**Keywords** Head and neck biopsies · Histology · Lab management · Quality improvement · Multi-level sectioning · Unstained slides

## Introduction

There is no universally accepted protocol for the initial cutting of slides for biopsy specimens. While the standard practice is to cut three hematoxylin and eosin (H&E) levels, some variation exists, and it remains institution specific. Slides for biopsy specimens at our tertiary care institution are cut according to different protocols depending on the nature of the tissue biopsied (i.e. the “part type”). For example, heart biopsies get 10 levels with every other level stained with H&E, and the rest saved as unstained slides,

while lung transplant biopsies get 8 levels with every other level stained with H&E and the rest saved as unstained slides. Other examples include gastrointestinal biopsies, which get two H&E levels and no upfront unstained slides. These practices have largely been based on intuition about quality practice rather than formal data on their need and efficacy. These differences are also more obvious when one is constantly reviewing referral slides from many other institutions, and one is amazed by the wide variation in biopsy handling practices. Some institutions cut three slides with 1–2 ribbons of 3–5 biopsy sections on each slide, whereas others cut only one slide with one section, occasionally putting ink-color-coded sections from more than one biopsy site onto the same slide.

A study was performed by Hameed et al. on prostate needle core biopsies comparing interval sectioning with upfront unstained slides versus using recuts when additional immunohistochemical studies are needed [1]. It showed that

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interval sections retained the focus of interest in a significantly higher proportion than recuts. This method was subsequently extrapolated and applied to biopsies from other sites at our institution, including head and neck, gastrointestinal, and other small biopsy specimens, without direct data to support its effectiveness and need. A follow up study was performed by Salaria et al. which showed that eliminating interval sectioning of GI biopsies and replacing them simply with two H&E levels resulted in financial benefits and increased job satisfaction among histotechnologists with no negative impact on the quality of patient care [2]. As a result, this method is in use today for gastrointestinal biopsies at our institution.

It is evident that not all biopsies should be sectioned using the same protocol as this might result in either loss of material, as is the case for prostate biopsies, or inefficient and wasteful practice, as seemed to be the case for gastrointestinal biopsies. Other biopsy sites lack data regarding best upfront sectioning protocols. In fact, we are not aware of any data in the literature from the head and neck. Currently, biopsies from the larynx, oral cavity, pharynx (oropharynx, hypopharynx, and nasopharynx), and sinonasal tract at our institution undergo interval sectioning with unstained slides cut upfront for potential ancillary studies. The unstained slides are stored until they are needed or until they must be discarded to create space for new slides. This results in a retention time of approximately 6 months. The aim of this project was to retrospectively evaluate the use of multi-level sectioning and upfront unstained slide cutting for biopsies from the head and neck to ascertain the most efficient practices while still maintaining quality patient care.

## Materials and Methods

After applying to the Human Studies Protection Office (HRPO) of Vanderbilt University Medical Center and approval of study as a quality project not subject to human studies approval, a search of the surgical pathology archives was conducted for head and neck mucosal-based biopsies only, using all of the following part type categories related to the head and neck available in our Copath system: ethmoid/sinus tissue, larynx biopsy, lip biopsy, nasal content, nasal mucosa biopsy, nose polypectomy, nose biopsy, odontogenic cyst, oral/mouth/epiglottis mucosa biopsy, oral mucosa, paranasal biopsy, sinus mucosa biopsy, skin/soft tissue/tongue biopsy, tongue biopsy, vocal cord/pharynx/trachea/palate biopsy, and tonsil biopsy. The results were grouped in the following categories for the purposes of this study: larynx, oral cavity, pharynx (oropharynx, hypopharynx, and nasopharynx), and sinonasal tract. A full 3 years of cases were utilized (1/1/2014 to 12/31/2016). Each block, as opposed to each case, was then treated as a separate data point for data

gathering since each block is a different piece of tissue cut independently from the other blocks. Non-biopsy cases that turned up in our search were excluded.

The following information was collected: date of accessioning, site of biopsy, diagnosis, number of total slides cut, number of slides stained with H&E, number of unstained slides saved, and number of unstained slides used for additional stains. For blocks with used unstained slides, the immunohistochemical or special stain performed was noted in order to ascertain the most frequent additional stain done on each tissue site.

Frequency of use of unstained slides was then calculated for the overall data, by year, and by site of biopsy. A 2 × 4 contingency table using the raw counts was constructed and a Chi square analysis was performed using an online statistical computation tool (<http://www.vassarstats.net>) to examine correlation rates and their statistical significance. More than 10% use of unstained slides was considered sufficient justification for continuing upfront unstained slide cutting for a particular anatomic site. For sites with less than 10% use, slides from cases for which an unstained slide had been utilized were pulled and re-reviewed by one pathologist (MSC) to see if diagnostic features were present on all levels cut and stained initially (specifically focusing on the last H&E stained section) to see if diagnostic information would have been lost had we only had the first H&E sections initially (without upfront unstained slides cut and kept) and had ordered the additional stains afterwards as needed.

## Results

There were a total of 1172 patients with 1479 parts and 1547 total biopsy blocks over 3 years (2014–2016). The blocks were distributed as follows: 706 (46%) from the larynx, 572 (37%) from the oral cavity, 184 (12%) from the pharynx, and 85 (5%) from the sinonasal tract. All of the cases had 5 initial slides cut, with either slides 2 and 5 stained with H&E or level 1, 3 and 5 stained with H&E, depending on the protocols which had been developed on an inconsistent “as you go” basis over the years. The remaining unstained slides had been held in the histology lab.

The overall use of at least one of the unstained slides across sites was 18.2% (Table 1). When stratified by site, there was a significant difference in the frequency of use of unstained slides ranging from 7.8% (55/706) for larynx, 21.9% (125/572) for oral cavity, 30.6% (26/85) for sinonasal tract and 40.8% (75/184) for pharynx (Chi square test,  $df=3$ ,  $p<0.0001$ ). For the larynx, this translated into 1378 unused slides stored in the laboratory over the 3 year time period. Each site was then stratified by year, and the rate of use of unstained slides was found to be relatively constant over time. Rates of use for 2014, 2015, and 2016 were 8.4%,

**Table 1** Usage rates for the unstained slides stratified by site and year (numbers are percentages followed by the raw number fraction in parenthesis)

	2014	2015	2016	All years
Larynx	8.4 (23/273)	8.3 (25/302)	5.3 (7/131)	7.8 (55/706)
Oral cavity	22.8 (39/171)	20.1 (39/194)	22.7 (47/207)	21.9 (125/572)
Nasal cavity and paranasal sinuses	42.9 (9/21)	22.6 (7/31)	30.3 (11/33)	30.6 (26/85)
Pharynx	40.6 (28/69)	39.7 (23/58)	42.1 (24/57)	40.8 (75/184)
All sites	18.5 (99/534)	16.1 (94/585)	20.8 (89/428)	18.2 (282/1547) <sup>a</sup>

<sup>a</sup>The difference between the sites for all years is statistically significantly different, Chi square 33.79 (df = 3),  $p < 0.0001$

8.3%, and 5.3% for the larynx, 22.8%, 20.1%, and 22.7% for the oral cavity, 42.9%, 22.6%, and 30.3% for the nasal cavity and paranasal sinuses, and 40.6%, 39.7%, and 42.1% for the pharynx. The most common stain ordered for the pharynx cases was p16 immunohistochemistry (59.7%) whereas for cases from the oral cavity (70.4%), sinonasal tract (35.1%) and larynx (74.5%), it was Grocott's methenamine silver (GMS).

Since the larynx had a frequency of use less than 10% overall, slides were re-reviewed from the ones where an upfront unstained slide was utilized. These 7.8% of larynx cases translated into 55 blocks, out of which 43 (78%) had slides available in the archives for review. All 43 of these parts/blocks showed that the lesion of interest, whether inflammatory or neoplastic, was present equally on all slide levels obtained. For two of the cases, the lesion was actually more obvious on the deeper levels/slides than on the first slide.

## Discussion

There is a paucity of data in the literature regarding the best approaches to histopathologic processing of biopsy specimens. Most of the studies that examine the diagnostic value of, or that provide a cost-benefit analysis of more extensive histopathologic examination of tissue, focus on the detection of micrometastases in sentinel (and other) lymph nodes, particularly in the setting of melanoma [3] and in carcinomas of the prostate [4, 5], oral cavity [6] and endometrium [7]. Other studies are more focused on efficient and high-quality histopathology lab processes, such as on the need for submission of sections of palatine tonsils and adenoids for histologic examination [8], the value of multiple levels for temporal artery biopsies [9], and the value of exhaustive searching for microcalcifications in stereotactic breast biopsies [10]. Few studies examine the utility of multilevel sectioning or the value of unstained slides cut upfront for efficiency and for assuring the presence of lesional tissue for ancillary stains, should they become necessary. This whole issue is little examined in the literature and probably most

examined for prostate needle core and temporal artery biopsies [1, 5, 9].

This study provides actual raw data on upfront multiple H&E slide cutting and unstained slide usage for head and neck biopsy specimens. These two procedures are designed to provide thorough, high quality, and timely evaluation of these specimens. This is widely practiced in surgical pathology laboratories but is almost wholly based on the notion of “this surely provides value and improved patient care.” This study more objectively addresses this main question and finds that the use of upfront unstained slides from most head and neck subsites was substantial enough to justify its continued use, at least using the subjective criterion of 10% or more slides utilization. However, for laryngeal biopsies, utilization of these unstained slides was less than 10%, which we deemed was low enough to question its utility. The other examined anatomic sites (pharynx, oral cavity, and sinonasal tract) had much higher rates of use (40.8%, 22.8%, and 26.2%, respectively).

The purpose of upfront unstained slides is to not lose the lesion of interest on deeper levels upon recutting the block for additional stains/studies. After investigating with our histotechnologists, we found that the levels are from consecutive slices of the paraffin block i.e. no tissue is lost in between one level and the next. Additionally, upon ordering subsequent recuts, the amount of tissue lost varies from none (the immediate subsequent section is taken for staining) to 9  $\mu\text{m}$  (3 paraffin slices are cut and discarded until an adequate slice is taken for staining) depending on the size of the tissue, its position in the block and the angle of the block holder on the microtome compared to the original angle used to cut the initial sections. To get a better idea if interval sectioning is preserving more lesional tissue, we undertook a review of the laryngeal biopsy cases where the unstained slides had actually been utilized. We found that the lesion of interest was present with no attenuation on the last level/last slide cut in all 43 of the examined parts. Given how we currently cut our biopsy specimens, whether unstained slides are cut upfront or the block is simply recut for more slides after the initial H&Es are reviewed, it is unlikely that the diagnostic lesion of interest would be lost. However, a

limitation of our study is that the cutting technique utilized for upfront and recut sectioning minimizes lost tissue by limited cutting between “levels”. Our results may not translate to those laboratories where step sectioning is more interval-based, with significant cutting between the levels generated.

Based on this study, we have eliminated upfront unstained slide cutting for laryngeal biopsies and moved to just two H&E slides at our institution (Fig. 1). GMS being the most common ancillary stain in laryngeal biopsies, this can easily be ordered on recuts with confidence that the organisms should be present on recuts. These changes save histology lab technologist time, save money, save storage space, and save technical effort and tracking. We also examined the most common stain performed by site and, as would be expected, found that p16 is the most common ancillary study done on pharyngeal biopsies. It is a well-established prognostic marker for oropharyngeal squamous cell carcinomas and is now recommended by the College of American Pathologists for all new oropharyngeal squamous cell carcinoma cases [11]. For the other three anatomic subsites, GMS was the most common special stain performed as an ancillary study because it is a cheap and easy method to evaluate for fungal infection when faced with inflammation and ulceration of unknown cause. The larynx is most commonly biopsied for dysplastic lesions and for squamous cell carcinomas, for which HPV testing is not recommended and for which special stains for organisms are not necessary.

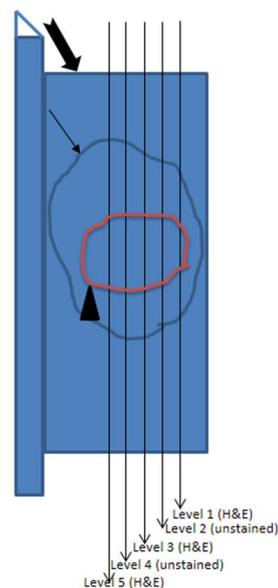
Our numbers also show that for each of the separate anatomic subsites, the rate of unstained slide use was constant from year to year over the study period (2014–2016). This consistent approach to biopsies in our institution gives the results internal and external validity as it allows us to safely assume that these numbers will also apply in subsequent years.

## Conclusion

This quality improvement study shows that multilevel sectioning with three H&E slides and upfront unstained slides from laryngeal biopsies may be unnecessary because the unstained slides are markedly underused compared to biopsies from other head and neck subsites. The relatively common use of upfront unstained slides from biopsies of the sinonasal tract, oral cavity, and pharynx appears to justify their being cut routinely, at least by the admittedly subjective cutoff of 10% utilized in this study. Even though changing practices at only one biopsy site may seem minimal, larynx biopsies are the most common type of biopsy from the head and neck overall in our practice. This study demonstrates that data-driven review of histology practices can provide support for special handling while showing where such practices may not be useful and can be discontinued. Laboratories are encouraged to evaluate histology sectioning

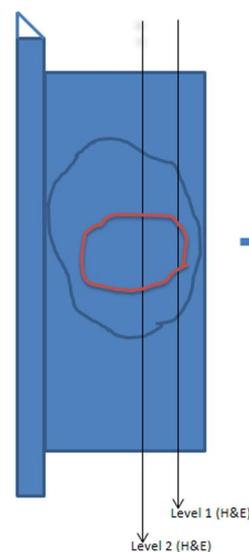
**Fig. 1** Schematic representation of the previous and revised sectioning methods for laryngeal biopsies showing no loss of diagnostic information when adopting the primary sectioning method as demonstrated by our data (thick arrow: formalin-fixed, paraffin-embedded tissue block; thin arrow: embedded laryngeal tissue; arrow head: lesion)

Current sectioning method

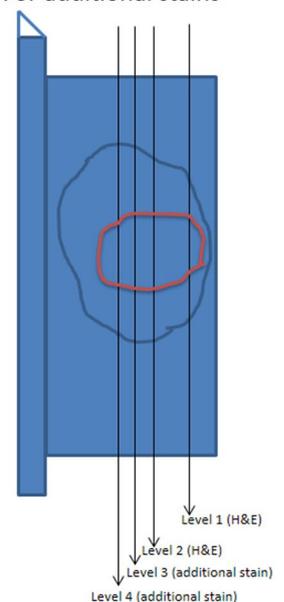


Proposed sectioning method

Initial sections



For additional stains



practices across all sites so that efficient, but still patient-centered and effective, practices can be followed.

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