Erroneous Patient Tissue Contaminants in 1574 Surgical Pathology Slides

Impact on Diagnostic Error and a Novel Framework for Floater Management

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***Context.*—Tissue contaminants on histology slides represent a serious risk of diagnostic error. Despite their pervasive presence, published peer-reviewed criteria defining contaminants are lacking. The absence of a standardized diagnostic workup algorithm for contaminants contributes to variation in management, including investigation and reporting by pathologists.**

***Objective.*—To study the frequency and type of tissue contaminants on microscopic slides using standardized criteria. Using these data, we propose a taxonomy and algorithm for pathologists on "floater" management, including identification, workup, and reporting, with an eye on patient safety.**

***Design.*—A retrospective study arm of 1574 histologic glass slides as well as a prospective study arm of 50 slide contamination events was performed. Using these data we propose a structured classification taxonomy and guidelines for the workup and resolution of tissue contamination events.**

***Results.*—In the retrospective arm of the study, we identified reasonably sized benign tissue contaminants on 52 of 1574 slides (3.3%). We found size to be an important parameter for evaluation, among other visual features including location on the slide, folding, ink, and tissue of origin. The prospective arm of the study suggested that overall, pathologists tend to use similar features when determining management of potentially actionable contaminants. We also report successfully used case-based ancillary testing strategies, including fluorescence in situ hybridization analysis of chromosomes and DNA fingerprinting.**

***Conclusions.*—Tissue contamination events are underreported and represent a patient safety risk. Use of a reproducible classification taxonomy and a standardized algorithm for contaminant workup, management, and reporting may aid pathologists in understanding and reducing risk.**

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Extraneous tissue fragments contaminating microscopic slides of patients to whom they do not belong represent a serious patient safety risk for diagnostic error in anatomic pathology. When not correctly identified, the extraneous contaminating tissue can lead to a diagnostic error and adverse patient outcomes. Identification errors, that is, when a mismatch occurs between the patient label and some or all of the tissue present, are among the most rapidly growing categories of malpractice claims involving pathologists.[1] Because of this, most laboratories have implemented extensive protocols to improve the labeling integrity of specimens, blocks, and slides during processing. However, these efforts do not address the problem of tissue contamination during processing but rather target specimen mix-ups and labeling errors. A large study of cross-contamination analysis comprising 275 pathology laboratories showed that between 98% and 99% of laboratories studied had written guidelines for reducing the occurrence of tissue contamination during processing.[2] However, the same study revealed that laboratories rarely have internal guidelines for identifying, tracking, or investigating contaminants, or "floaters," once they occur. Only 6.1% had written protocols for documentation of extraneous tissue in surgical pathology reports, 5.7% had guidelines for removal of extraneous tissue from blocks, and 4.7% had protocols for handling extraneous tissue on microscopic slides. In 24% of laboratories, no comment or record was ever kept to document extraneous tissue.[3] This lack of standardized measurement, workup, or reporting represents an important obstacle in assessing the effectiveness of quality improvement measures.

A proposed tissue contamination pathway can be classified into 2 recognizable forms. First, thin sections of tissue cut by a microtome can transfer to glass slides,

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typically at processing steps involving slides being immersed in liquid, either to pick up freshly cut tissue ribbons or while slides are being stained in reagent baths.[4–6] Such slide contaminants, or floaters, are present only on a single glass slide and not in the original paraffin tissue block. Specific morphologic features are associated with this type of contaminant, such as abnormal tridimensional folding, a position outside the primary plane of focus, or a location away from the primary tissue or at the periphery of slides.

Second, *carryover* contaminants are embedded within the primary paraffin block itself. Carryovers are believed to originate at or before the point of tissue embedding and have traditionally been attributed to either the grossing bench or the embedding station[4] (or even to the clinical procurement of tissue, for example the frequent presence of duodenal mucosa in a stomach biopsy from an upper endoscopy procedure). Identification of a carryover contaminant requires careful examination of morphology and a review of accessioning, grossing, and embedding logs to determine the origin of the contaminant, as well as correlation with clinical history and radiologic findings. Molecular methods to establish the exogenous nature of the tissue can be used, but tissue preservation and depletion are important concerns during any step-by-step investigation.

Previous studies investigating the prevalence and significance of contaminants on microscopic glass slides often grouped floaters and carryovers together. Because of the challenges of studying a nonstandardized area of health care delivery, these studies often showed results with poor reproducibility. For example, a study of 378 840 total slides by Gephardt and Zarbo[2] revealed a prevalence of contaminants that varied between 0.6% and 2.9%, depending on the study method used. In their corresponding prospective study, slide contamination ranged from 1.8% to 0%, with laboratories above the 90th percentile reporting no cases of slide contamination at all. A different study reported a prevalence ranging from 0.01% to 1.2% (a 100-fold difference) within the same laboratory, depending on the criteria used for the identification of contaminants.[3] In a study assessing the prevalence of contaminants originating from the water bath and slide stainer, floaters were identified on 25% of blank slides when screening for contaminants of any size and type, but on only 3% of tissue sections when using different, more restrictive identification criteria.[5] A proposed explanation from the study authors was that different laboratories differ in "cleanliness" for different processing steps.[2] We suggest that taken together, prior efforts to produce generalizable, reproducible, and practical information on microscopic slide contamination highlight that the measured prevalence of contaminants is highly dependent on the identification method and criteria used, and that the lack of standardization in nomenclature and investigation complicates safety event measurement.

In a majority of pathology laboratories, the identification of a tissue contaminant is the responsibility of the pathologist. This is typically done in a 2-step process. First, candidate tissue contaminants need to be recognized on the microscopic slides, often based on visual characteristics judged relevant or risky by the pathologist. Then, a decision must be made regarding whether to further investigate or ignore the candidate contaminant tissue. Pathologists may vary in their opinions regarding features considered suggestive of contamination and what requires further investigation or reporting. Here we propose that the use of standard guidelines and an established nomenclature in the identification of slide contaminants could contribute to creating more generalizable, reproducible, and practical information for pathologists and laboratories. The establishment of standardized criteria could also assist pathologists in adopting an algorithmic approach to contaminant decision-making, and could contribute to improved consistency in reporting, investigation, and resource management.

The objectives of this study were to investigate the criteria used by pathologists at Beth Israel Deaconess Medical Center (Boston, Massachusetts) in the identification of contaminants, establish consensus on key elements for contaminant investigation and reporting, and retrospectively assess the prevalence and types of contaminants on microscopic slides of our institution. Based on this analysis, we then propose a novel contaminant classification system and a list of criteria for contaminant investigation and resolution in a busy academic surgical pathology laboratory practice.

MATERIALS AND METHODS

Institutional Review Boards and Protection of Human Subjects

The authors obtained an institutional review board waiver from the Committee on Clinical Investigations of Beth Israel Deaconess Medical Center, deeming this research to be nonhuman subject research following institutional review board review (protocol approval number 2022D000616).

Survey and Aggregation of Contaminant Investigation Criteria

We surveyed practicing pathologists at our subspecialized academic institution regarding the visual features routinely used to recognize potential tissue contaminants on microscopic slides, as well as the features guiding their decision-making when resolving candidate contaminants. Some pathologists were surveyed in person during routine service or based on availability and others were surveyed by email. Only pathologists covering subspecialty services where slide contaminants were expected to be routinely encountered were surveyed (gastrointestinal, genitourinary, gynecologic, thoracic, head and neck, liver, hematopathology, cytopathology, dermatopathology, bone and soft tissue, and renal). Several fellows were also surveyed. Overall, the response rate was 16 of 32 pathologists surveyed (50%), and included responders of all levels of training (in training and new in practice up to several decades of experience), with prior training in multiple different institutions. The pathologists' answers were compiled, and similar answers were categorized according to screening, inclusion, and exclusion factors (Supplemental Table; see supplemental digital content).

Retrospective Study Arm and Contaminant Nomenclature

The presence of slide tissue contaminants (floaters) and block tissue contaminants (carryovers) was retrospectively reviewed in 1574 histologic glass slides cut during the course of routine clinical practice from 879 blocks belonging to 185 cases. First, 1374 consecutive slides belonging to signed-out cases were selected by date of accessioning, beginning 6 months before the start of our study. This group was felt to be large enough to recapitulate the distribution of cases routinely processed by our institution. One hundred more consecutive slides were picked from a period of time beginning 1 year before the study and 100 more from a period of time beginning 3 years before the study, in order to control for year-to-year variation; no significant variation was found. Slides featuring tissue with suspicious features (location on slide, ink, folding, tissue of origin), as determined by our departmental survey, were labeled as candidate contaminants. Paraffin-embedded blocks of cases with candidate contaminants more than 0.1

mm in maximal dimension were visually examined to determine the presence of the tissue within the block. When available, subsequent profiles and step-level sections were also examined. Contaminants visible on a glass slide, but not present in subsequent sections or within the block, were labeled as floaters. Contaminants were deemed definitive carryovers when they were found within the corresponding block and/or in subsequent sections, yet were incompatible with the specimen of interest (tissue from different anatomic site, marking ink present when not noted on the original specimen, and/or of alternative color).

Prospective Study Arm and Contaminant Identification Technique

We sought to better understand the relationship between the features of contaminants and the identification steps used by pathologists to resolve a case. We were especially interested in correlating the characteristics of suspected contaminants with the method ultimately successful in resolving a case. To achieve this, all pathologists at our institution were asked to self-report suspected contaminants identified during routine service. Every reported candidate contamination event was reviewed until a total of 50 cases were obtained (covering an approximate period of 8 months). Our data included contamination events originating from varied pathology subspecialty services (breast, gastrointestinal, genitourinary, gynecologic, thoracic, liver, hematopathology, dermatopathology, and cytopathology). During this time period, there were no reported cases originating from the renal or bone and soft tissue services. The decision-making process used in the identification of reported floaters was assessed. The Pearson χ^2 test of independence was used to assess the statistical significance between the observed features and the methods used to resolve cases when the expected number of values for each cell being compared was at least 5. When the expected number of values was less than or equal to 5, a Fisher exact test of independence was used instead. We hypothesized that contaminants with different characteristics may benefit from being worked up differently and that subclassifying contaminants based on certain features could support improved laboratory guidelines for management and reporting.

Statistical Analysis

Because an important method in our study was the assessment of the presence of fragments of incongruous tissue within paraffin blocks, and because the most direct method to assess this was by visual inspection of subsequent sections taken deeper within the block, we sought to calculate the probability of a piece of tissue being present in a subsequent profile or section based on the observed maximal dimension of the tissue on the initial slide. We used a modified system of integral geometric probabilities based on the Buffon-Laplace theorem (supplemental digital content). We calculated that tissue fragments of observed maximal dimension equal to or larger than 102 μm have an expected threshold of 95% likelihood to be present in a section taken 25 μm deeper into the block (the depth of a routine additional level section at our institution). Based on this observation and analysis, and to account for inefficiencies in the slide preparation process, we made the decision to suggest 0.1 mm as a minimal size criterion for the investigation of slide contaminants.

RESULTS

Prevalence of Slide Tissue Contamination

A representative cohort of 1574 histology slides originating from 879 tissue blocks with no previously documented tissue contaminants was reviewed after being obtained from departmental archives. Suspicious features associated with tissue contamination in our survey (Supplemental Table) were used to identify candidate slide contaminants both inside and outside the area of the paraffin-embedded tissue ribbon on each slide. Every slide exhibited some minute degree of microscopic slide contaminant, and showed at least one cell or fragment of *debris* located outside the boundaries of the paraffin-embedded tissue ribbon on the slide (Figure 1, A), a feature of floater-type contaminants. However, the definitive identification of candidate floaters proved to be increasingly difficult below an observed size of 0.1 mm by visual examination alone. This supported the expectations laid out in our statistical geometric probability study (supplemental digital content), which suggested a threshold of 102 μm for an embedded tissue fragment to have a probability of at least 95% of being present within a routine additional section taken at a depth of 25 μm (routine deeper section at our institution). Consequently, we decided to adopt a minimal size of 100 μm as criterion for evaluation of candidate floater or carryover moving forward in our study. Tissue below that size was categorized as debris and investigated in a different manner. Candidate contaminants that lacked specific features (such as fragments of acellular, amorphous, or pluricellular fibroconnective tissue) also proved inadequate for advanced investigation techniques and were categorized as debris. However, it is important to note that incompatible ink (ie, ink seen where no ink was reportedly used, or ink an alternative/wrong color) was observed on some debris fragments of various nature and size (Figure 1, B).

Slide contaminants (floaters) larger than 100 μm were conclusively identified in 47 of 1574 total slides (3.0%) by an absence from both additional sections and the associated paraffin-embedded block (Table 1). Larger floaters (>0.25 mm) were present in 11 of 1574 slides surveyed (0.7%) and were less frequent than smaller ones (≤0.25 mm), which were present in 36 slides (2.3%). Block contaminants (carryovers) were conclusively identified on 5 of 1574 slides (0.3%), predominantly because of a recognizable difference in organ of origin of the carryover and that of the specimen of interest. Additional candidate carryovers embedded within the block and presenting with at least one surveyed feature suspicious for contamination (Supplemental Table 1), but no incompatibility in anatomic site of origin and no final impact on the pathologic diagnosis, were observed on 252 of 1574 slides (16.0%). However, these 252 candidate carryovers could not be conclusively recognized as true contaminants without additional cost-intensive identification methods, such as molecular or cytogenetic techniques. Because of the large number of candidate carryovers and because of their predominantly benign and clinically unimpactful nature (most were composed of tissue compatible with the section of origin), a systematic investigation of every candidate carryover by advanced techniques proved impractical and was not performed. Only one candidate contaminant (carryover) was positive for malignancy. However, the same type of malignant tissue was also present in a different block of the same specimen from the same patient. Consequently, this likely endogenous contaminant had no impact on the final diagnosis.

Endogenous Tissue Mimicking Contaminants at Slide Periphery

In our retrospective study, the use of the distance between a candidate contaminant and the slide's section of interest revealed the frequent presence of multiple specific loose, irregularly folded fragments of tissue presenting as 1 or 2 thin parallel lines. The parallel lines were always located at opposite sides of the glass slide and disconnected from the main section of interest (Figure 1, C). This type of candidate contaminant was always embedded within the block and

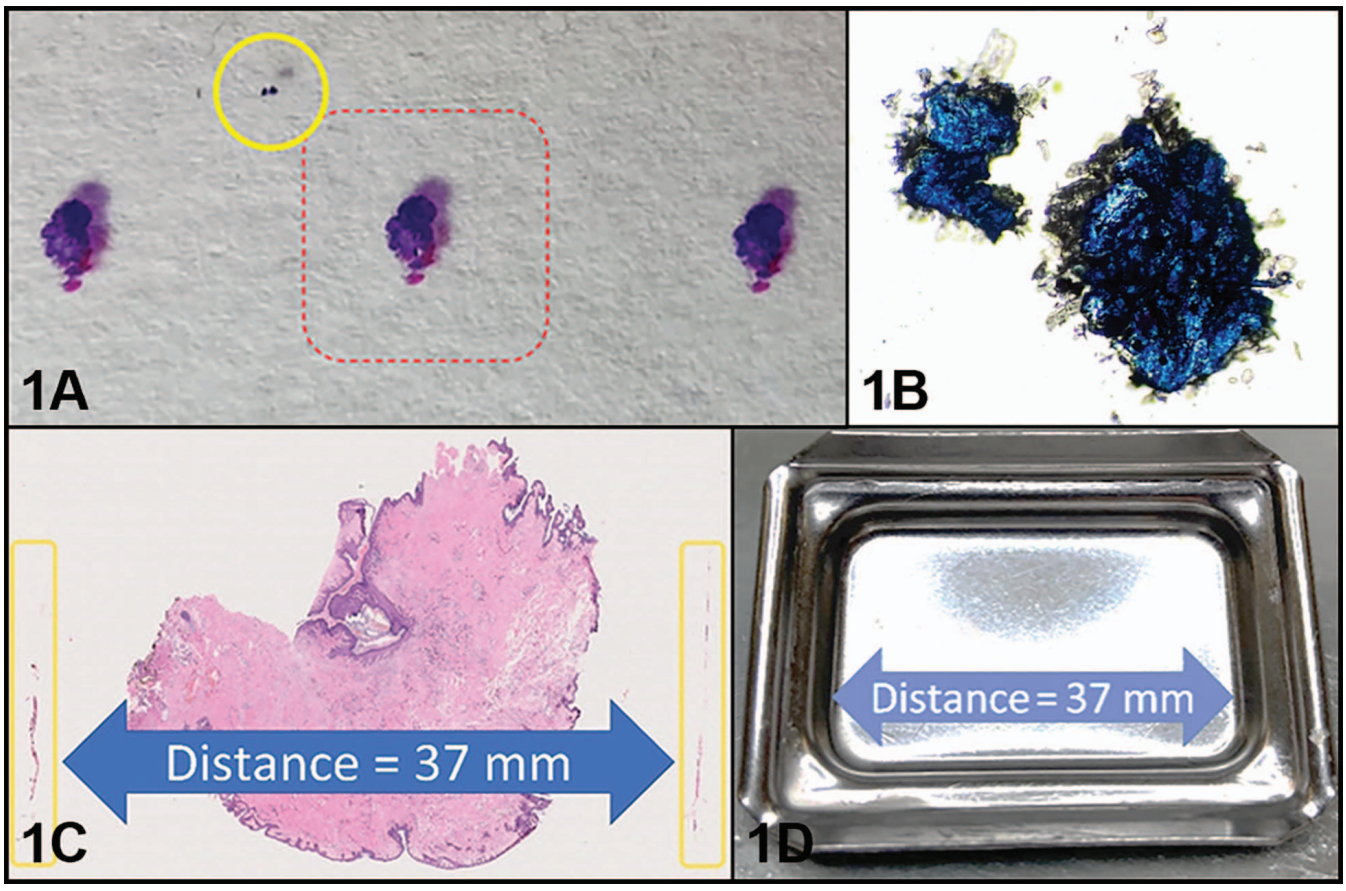

Figure 1. *A, Contaminating tissue (yellow circle) confirmed to be a floater (not present on multiple profiles or slides or in the block) located outside the boundaries of the paraffin block's embedding mold (dotted red lines). B, Small fragment of acellular tissue with inking incompatible with the case of interest as well as characteristic 3-dimensional features of tissue contaminants. C, Surgical glass slide with linear, folded, and fragmented tissue aggregated at both edges of the slide, corresponding in location and dimensions to friable endogenous tissue accumulated at the edges of the embedding mold (D) during the creation of the paraffin block.*

compatible with the section of interest, despite showing prominent folding. The compatibility with the section of interest was demonstrated by immunohistochemistry. When lines were present in pairs, our investigation showed that the exact distance between the 2 lines corresponded to the length of the specific metallic mold used for the paraffin-embedding processing step of the tissue (Figure 1, D).

Preferred Contaminant Identification Techniques

In order to study the step-by-step process of identifying and reporting floaters and carryovers, we requested that pathologists at our institution self-report suspected contaminants identified during a period of 8 months. In total, 50 cases were identified and subsequently reviewed. Criteria used to guide the sequence of identification steps and decision-making were combined into subcategories and correlated with the final identification technique used to resolve the event (Table 2).

Pathologists reported an equivalent number of floaters and carryovers (25 versus 25). Visual inspection alone was used to resolve 33 of 50 cases (66%). This was more likely to represent a satisfactory solution for floaters (21 of 25 cases; 84%) compared with carryovers (12 of 25 cases; 48%), $P = .007$. Conversely, carryovers were more likely to require additional and advanced workup steps as opposed to floaters. This suggests that floater contaminants could often benefit from a different, more limited, and less resource-intensive approach than carryover contaminants. This

Table 1. Review of 1574 Pathologic Slides (879 Blocks, 185 Cases) Using Consensus Screening Criteria

			No. (%) of Slides Showing Any Visual Element Suggestive of Contamination[a]				No. (%) of Slides Showing Definitive Floaters			No. (%) of Slides Showing Definitive Carryovers		
Type	Blocks	Slides	<0.1 mm	Any >0.1 mm	0.1–0.25 mm	>0.25 mm	Any >0.1 mm	0.1–0.25 mm	>0.25 mm	Any >0.1 mm	0.1–0.25 mm	>0.25 mm
All	879	1574	1574 (100)	252 (16.0)	181 (11.5)	71 (4.5)	47 (3.0)	36 (2.3)	11 (0.7)	5 (0.3)	3 (0)	2 (0.3)
Biopsy	221	676	676 (100)	93 (13.8)	56 (8.3)	37 (5.5)	16 (2.4)	11 (1.6)	5 (0.7)	1 (0.15)	1 (0.15)	0 (0)
Large section	658	898	898 (100)	159 (15.2)	125 (11.9)	34 (2.1)	31 (3.5)	25 (2.8)	6 (0.7)	4 (0.45)	2 (0.3)	2 (0.3)

[a] Supplemental digital content, Supplemental Table.

Table 2. Review of 50 Pathologic Cases With Contaminants Identified by the Pathologist and the Techniques Used to Obtain Satisfactory Identification

Ultimate Identification Method Used, No./Total (%) (N = 50)	Contaminant Taxonomy, No./Total (%)		Histologic Compatibility With Specimen and Procedure Site, No./Total (%)		Dysplasia or Malignancy Present, No./Total (%) (n = 24)	Significant Clinical Impact and Patient Safety Concern, No./Total (%) (n = 10)
	Floater (n = 25)	Carryover (n = 25)	Discordant With Organ or Site of Procedure (n = 28)	Concordant With Organ or Site of Procedure (n = 22)		
Visual inspection alone 33/50 (66)	21/25 (84)	12/25 (48)	16/28 (73)	17/22 (61)	11/24 (46)	0
Review of additional sections or examination of the block 4/50 (8)	3/25 (12)	1/25 (4)	3/28 (14)	1/22 (4)	3/24 (6)	0
Interdepartmental communication and coordination 10/50 (20)	1/25 (4)	9/25 (36)	9/28 (41)	1/22 (4)	7/24 (29)	7/10 (70)
Karyotype/XY FISH 2/50 (4)	0	2/25 (16)	0	2/22 (7)	2/24 (8)	2/10 (20)
FISH fingerprinting (multiplex STR) 1/50 (2)	0	1/25 (4)	0	1/22 (4)	1/24 (4)	1/10 (10)

Abbreviations: FISH, fluorescence in situ hybridization; multiplex STR, DNA fingerprinting by multiplex short tandem repeats; XY FISH, FISH analysis of X and Y chromosomes.

supports the idea of performing an early differentiation between floaters and carryovers in the investigation of candidate contaminants. Cases showing tissue discordant with the anatomic site of the procedure but no likelihood of metastatic origin (examples are benign tissue and incompatible sexual organ tissue such as uterine tissue in a male patient) were not investigated using complex additional identification methods such as cytogenetic or molecular techniques (0 of 28). Only fragments showing a tissue type concordant with the organ and/or the site of procedure ultimately required advanced identification methods (3 of 22 cases; 14%) compared with cases discordant with the organ and/or site of procedure (0 of 28 cases; 0%); however, the number of cases was insufficient to achieve statistical significance ($P = .10$). Cases showing a candidate contaminant that was discordant with the site of the procedure were most likely to be resolved by identifying the case of origin through intradepartmental communication and coordination and by reviewing processing or grossing logs (9 of 28 cases; 41%) compared with cases showing tissue that was concordant with the organ or the site of procedure (1 of 22 cases; 4%), $P = .02$. Finally, pathologists were significantly less likely to determine the nature of a floater by visual inspection alone and were more likely to use more advanced techniques when the origin of the floater demonstrated clinical significance or an impact on the diagnosis in the final pathology report (10 of 10 cases; 100%) compared with cases with no clinical impact (3 of 40 cases; 7.5%), $P < .001$.

DISCUSSION

Specimen cross-contaminants are a major patient safety concern for surgical pathology laboratories and create a risk of adverse events for patients. When present on a surgical pathology glass slide, contaminants can be mistaken for endogenous/patient-specific tissue and create misidentification and diagnostic error. Laboratories are committed to devoting resources to minimize the incidence of cross-contamination. However, in the absence of measures of the true prevalence of tissue contaminants, the effectiveness of the measures taken is difficult to quantify in a reliable manner. In other words, the key quality and safety principle "If you don't measure it, you can't manage it" applies.

Because identifying slide contaminants is generally the responsibility of the pathologist, both factors related to the pathologist's recognition of possible contaminants on slides and elements factoring in the pathologist's decision-making process for the investigation of suspicious tissue will have an impact on the prevalence of slide contaminants ultimately reported by a laboratory. However, comprehensive standardized guidelines were not found during our review of the literature. This provides a possible explanation for the discrepancies among previously published prevalence studies reported by institutions using different selection and identification criteria for slide contaminants. Additionally, this highlights the potential benefits of a standardized nomenclature and management guidelines that are generalizable across laboratories.

Most Tissue Contaminants Go Unreported

Our findings of 47 definitive floaters and 5 carryovers in a retrospective investigation of a cohort of departmental slides with no previous mention of contaminants in the corresponding pathology reports (Table 1) suggests that a significant proportion of true tissue contaminants present on slides might not be reported after routine pathologic examination. It is possible that a number of slide contaminants are simply missed during visual inspection. However, another possible explanation is that the routine identification of floaters and carryovers might not always be perceived to be necessary by pathologists or laboratories in the presumed absence of potential clinical impact (or impact on the associated pathology report). Pathologists may also feel that

they have no standardized, useful, or meaningful (or efficient) quality and safety methodology by which to report tissue contamination. Without a nomenclature for significant or nonsignificant contaminants, busy pathologists are likely to dismiss what they perceive to be nonsignificant contaminants. This was reflected in our retrospective study by the fact that none of our identified contaminants were determined to have a clinical impact upon review of their respective cases. Another likely contributing factor is that it might prove impossible or excessively costly to pursue the definitive identification of every tissue fragment exhibiting suspicious features during routine pathology. This was supported by our analysis, which identified at least 252 suspicious fragments of tissue in 1574 slides, some of which were poor candidates for commonly used advanced identification techniques. This suggests that the true prevalence of slide floaters, carryover, and debris might be significantly underestimated by visual inspection alone in our laboratory, and this is likely true in other laboratories as well. This finding may in part explain the discrepancies observed between retrospective and prospective studies previously published in the literature (ie, prospective rates have tended to be lower). If true, this finding highlights the need for better-defined guidelines in selecting which tissue fragments to analyze and report. This is especially true when pursuing the objective of comparing the prevalence of cross-specimen contamination between laboratories, which reinforces a need for a standardized taxonomy and workup of potential tissue contaminants. Finally, a major tenet of the patient safety movement is measuring and studying near miss and no-harm events to understand and reduce risk rather than focusing only on harmful events, which when applied to tissue contamination yields a wealth of missed opportunities to understand risk and improve processes. For example, a contaminant from one part of a case to another could be clinically insignificant (benign endometrium, for example) or could have critical cancer staging implications (carryover of malignancy from one anatomic site to another). The former, if dismissed without reporting or study, represents a critical safety signal that the authors propose should be measured in any laboratory concerned with safety and reliable clinical activities. To reinforce the need for analysis, the 2 events above share the same root causes and have a different outcome only because of chance, that is, a true near miss or close call event, constituting a great opportunity for learning.

The Need for a Minimal Size Cutoff in Defining and Studying Tissue Contaminants

In our retrospective cohort, smaller candidate slide contaminants were shown to be more prevalent than larger ones (Table 1). However, smaller fragments of tissue and cellular aggregates also proved increasingly difficult to identify as definitive floaters or carryovers by visual examination alone, making the true prevalence of exogenous contaminants difficult to determine without the use of resource-intensive identification techniques. Moreover, as the cutoff dimension approaches the size of a single cell, the number of candidate contaminants becomes incalculable, and the quantity of tissue available for each candidate contaminant becomes insufficient for definitive identifications of floaters (by examining the block; see supplemental digital content) and carryovers (using advanced molecular techniques that require a minimum quantity of tissue). These findings suggest that a relationship exists between the prevalence of floaters and their size, with smaller floaters being more prevalent on slides but also generally more difficult to fully investigate. Our retrospective investigation also revealed a high number of candidate contaminants lacking traditional identifying features, such as loose fragments of nonspecific tissue (fibroconnective tissue, amorphous aggregates, acellular elements) that could not be investigated with advanced techniques by pathologists. This overall phenomenon supports the utility of categorizing certain candidate contaminants as debris (defined as tissue of unknown origin with insufficient size, scant cells, or identifying features inadequate for further investigation). The fact that several items of debris in our study were unequivocally shown to be extraneous in nature by the presence of incompatible inking suggests that an indeterminate number of minute and/or acellular carryovers represent true cross-specimen contamination. Taken together, our results highlight the utility of integrating standardized minimal size and minimal identifiable features criteria in contamination studies, especially when a goal is to generate generalizable results. Obviously, when debris is excluded from contamination studies, the true prevalence of cross-contamination events may be underestimated, but the authors respectfully propose that a boundary must be set to reproducibly and practically study contamination events.

Contamination Pitfall: Artifact Introduced by Processing Can Mimic Tissue Contamination Events

Our review of visual characteristics routinely associated with contaminants by our pathologists showed that slide tissue fragments with folding, drying, and a peripheral location were frequently due to common artifacts created by the deposition of endogenous tissue/debris at the edge of the embedding mold during the creation of the paraffin block, and did not represent true cross-contamination events. This finding highlights that using certain characteristics such as a peripheral location or suspicious folding or drying pattern (Figure 1, C) as screening tools for contamination may not be specific because of routine pitfalls. The finding also highlights potential benefits for pathologists in being familiar with commonly encountered mimics of tissue contaminants. Distinguishing visually between true contaminants and this artifactual phenomenon observed by the authors is embedded in the proposed tissue contamination algorithm, which the authors describe below.

Ancillary Testing as a Method for Tissue Contaminant Workup

An additional goal of our review was to determine which molecular techniques were favored as advanced identification techniques of complex cases. In our cohort, sex chromosome investigation by fluorescence in situ hybridization was the most popular first step (Figure 2, A and B) and DNA fingerprinting by multiplex short tandem repeats was the favored final step. The advantages of sex chromosome investigation by fluorescence in situ hybridization were minimal tissue consumption and a rapid turnaround time, whereas the main disadvantage was that only tissues with known differences in sex chromosomes (ie, suspected patients of different biological sexes) can be differentiated. The advantages of DNA fingerprinting were specificity and the commercial availability of multiple different techniques (multiplex short tandem repeats were favored in our cohort). The disadvantages of DNA

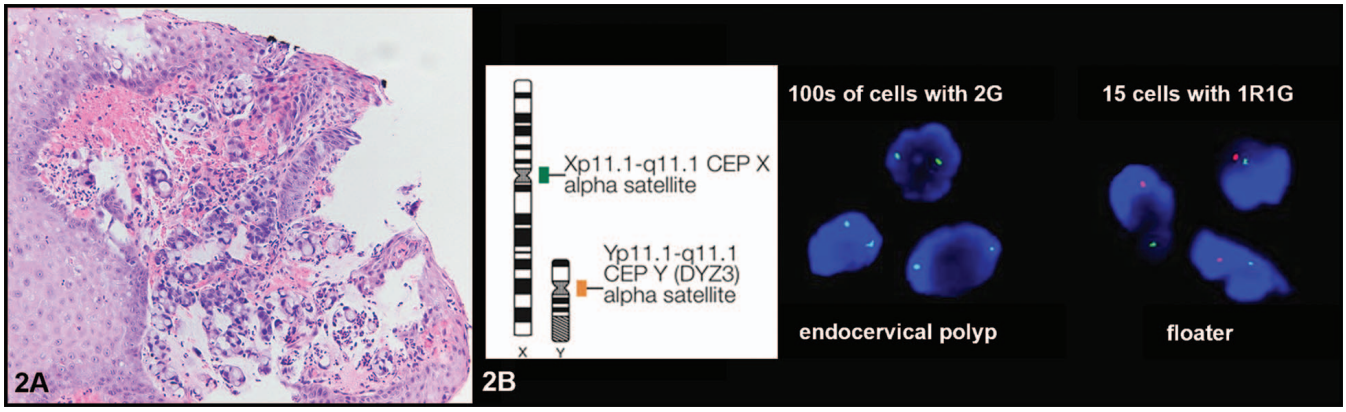

Figure 2. *A, Malignant signet ring cells observed on an endocervical polyp. B, Fluorescence in situ hybridization analysis of X and Y chromosomes using CEPX (spectrum green) and DYZ3 (spectrum red) probes targeting specific regions of chromosomes X (Xp11.1-q11.1) and Y (Yp11.1-q11.1) was used to confirm the exogenous origin of the malignant signet ring cells as contaminants, with minimal tissue consumption (hematoxylin-eosin, original magnification ×100 [A]).*

fingerprinting were that it required referral to an outside reference laboratory and that it required large amounts of paraffin-embedded tissue for analysis. In our cohort, DNA fingerprinting often exhausted the available tissue of the submitted case, which precluded further characterization or clinical testing if needed.

Pathologists Already Use Loose Features for Contaminant Workup Decision-Making

Our prospective study of 50 contaminant identification events showed that certain features of contaminants were associated with the decision-making steps used by pathologists to resolve their cases. Floater contaminants were significantly more likely than carryovers to be resolved based on visual features directly at the microscope or by the examination of additional sections and/or the block (Table 2); this was true for floaters of any degree of clinical significance. In contrast, carryovers embedded in the paraffin block were more likely to require intradepartmental coordination and advanced techniques; they were the only type of contaminant that required advanced molecular methods. Our results also indicated that all cases identified as debris were not considered for workup. This supports the idea that contaminants devoid of significant diagnostic or clinical implications are often resolved visually by the pathologist. Taken together, these findings support that there are benefits in performing early differentiation between debris, floaters, and carryovers in the investigation of candidate contaminants. It also suggests that debris and floater contaminants could benefit from a different, more limited, and less resource-intensive approach to identification than carryover contaminants.

By integrating the pathologists' observed actions in managing contamination, and prevalence and classification of tissue contaminants with general patient safety and quality principles centered around standardization and detection of risk, the authors developed a management algorithm as described below.

Practical Decision-Making for The Pathologist: A Proposed Algorithm

Here we propose a step-by-step guide to identify candidate contaminants according to the contaminant taxonomy and institute further workup where appropriate (Figure 3). Finally, the authors share a template for routine reporting of contaminants by standard molecular techniques (supplemental digital content).

Limitations

A limitation of this study is the small size of both our retrospective cohort (1574 slides) and our prospective study of contaminant identification events (50 cases) in comparison with the total number of slides produced annually by our institution. This limitation exists in part because of the significant time investment required to initially screen for floaters as small as a few cells, and in part because of the low number of contaminants that are routinely identified and reported in our laboratory. An additional limitation of our study is that our reviewed cohort may not be representative of the type of slides investigated by other laboratories. However, when using conservative floater screening criteria (definitive floaters and carryovers combined, >0.1 mm), our reported prevalence (3.3%) was in line with the prevalence (2.9%) reported by a prior large-scale study of 275 laboratories conducted by the College of American Pathologists,[2] leading us to believe that our sample is representative and can be reasonably generalized.

Another limitation of the investigation is that tissue identified as a floater, meaning a contaminant that does not originate from the paraffin block, could still have transferred onto the slide from the same overall case. This cannot be ruled out without using molecular techniques for complex cases with compatible histology, and is something that the pathologist should consider when evaluating contaminants with clinical or pathologic impact.

Finally, because pathologist participation was voluntary, there may be some special factors about engaged pathologists that make their assessment of potential contaminants different from that of a cohort of pathologists mandated to participate. The 50% participation rate we observed is higher than typical reported survey participation rates.[7] Although participation was voluntary, our cohort represented pathologists of mixed levels of experience, seniority, and subspecialty expertise.

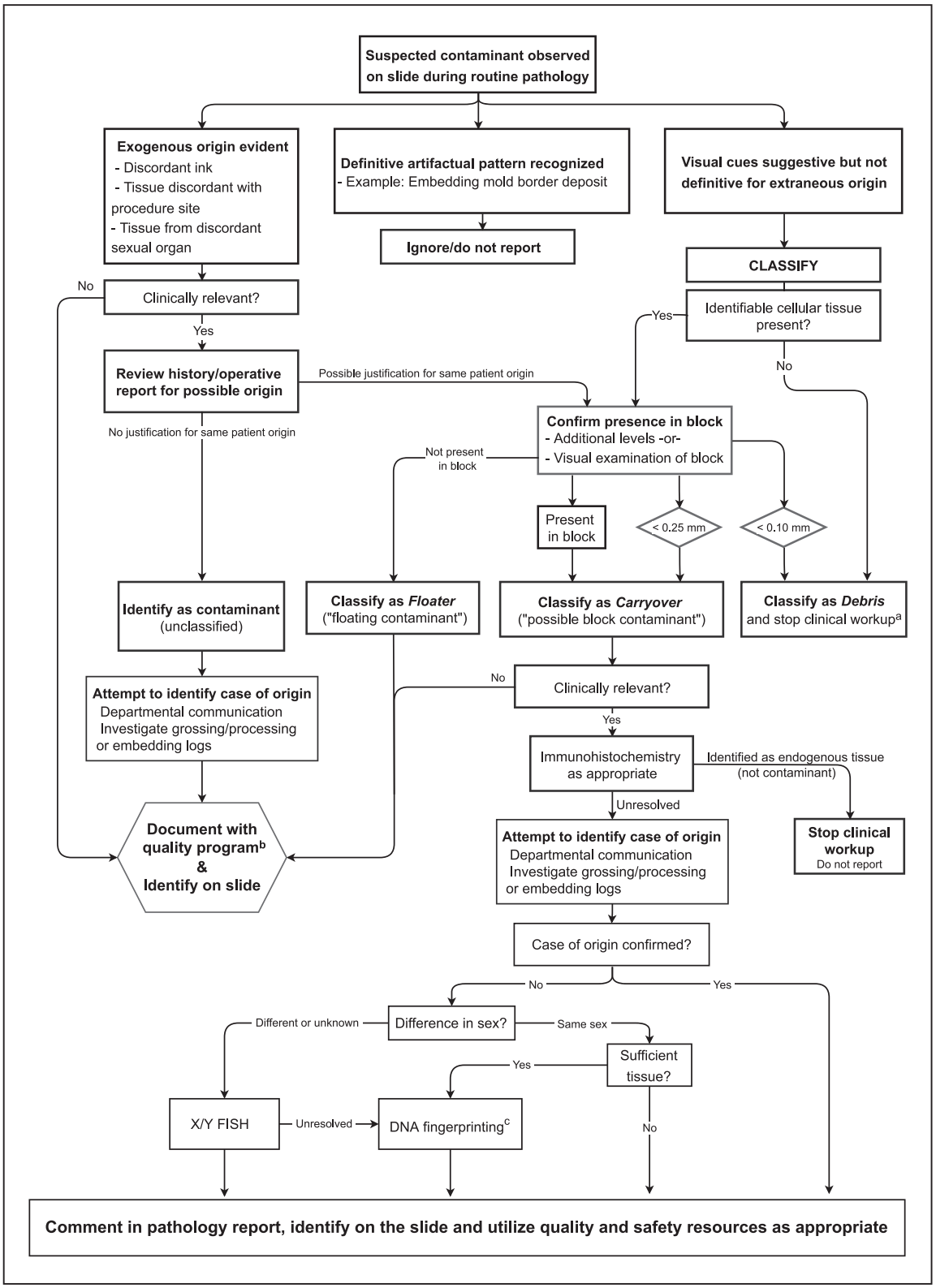

Figure 3. *An algorithm proposed to aid in the classification and workup of potential tissue contaminants. First, the pathologist flags a fragment as a potential contaminant. Then, visual attributes and the presence or absence of tissue in the block determine whether the contaminant is classified as a false contaminant mimic, inconsequential debris, floater, or carryover. Finally, a detailed algorithm is proposed for the subsequent workup of cases where tissue contamination is suspected, up to and including ancillary techniques if necessary. Documentation and internal quality and safety protocols may differ by institution. Abbreviation: X/Y FISH, fluorescence in situ hybridization analysis of X and Y chromosomes.* [a]*Pursue workup in rare clinical context when acellular, single-cell, or minute cellular tissue contamination may be clinically significant (sentinel lymph nodes, forensic tissue identification specimens, etc).* [b]*Documentation based on institutional protocol, and as appropriate if a departmental database or quality improvement initiative is in place. Although clinical workup can be discontinued, these safety events should be documented, measured, and investigated to determine common root causes and improve laboratory practices.* [c]*Multiple different techniques can be used. Multiplex DNA short tandem repeat analysis is favored as the initial step.*

CONCLUSIONS

Taken together, our results suggest that cross-specimen contaminants and especially contaminants of small size are far more common than previously reported. Our investigation supports the notion that the criteria used in the screening and identification of contaminants have a significant impact on their reported prevalence. Furthermore, our findings support the idea that the creation of a standardized taxonomy and classification system could generate benchmark data regarding slide tissue contamination that could help national surgical pathology quality leaders to improve and validate local quality practices.

Furthermore, as every contaminant is representative of a laboratory contamination event, and because laboratory contamination events are an important source of identification error leading to patient harm, surgical pathology and quality laboratory leaders would be wise to consider the benefit of systematic reporting to detect contaminant risk, especially because the impact of these type of contaminants on ancillary molecular studies has so far not been well characterized.

Finally, the authors hope that the aggregation of our findings into a practical algorithm for specimen contamination taxonomy and management will be useful to dedicated patient safety and surgical pathology leaders as they navigate the ongoing safety risk of an inherently messy surgical pathology practice.

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