Risk of Misdiagnosis Due to Tissue Contamination May be Higher for Certain Specimen Types

Changes to laboratory staining techniques offer opportunity to reduce contamination events

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Preface

Tissue contamination in the anatomic pathology lab has long been accepted as a part of the working environment. While laboratories strive to reduce contamination as much as possible, it has been viewed more as an inconvenience than as a serious issue.

...even in instances where the risk of misinterpretation is unlikely, loss of productivity as a result of contamination is a serious issue.

But that view is changing. As this white paper demonstrates, the risk of misinterpretation may be greater than most pathologists currently realize, especially in certain tissue types. Also, this paper illuminates a source of significant contamination that has largely been overlooked – staining baths.

Patient safety demands that laboratories take every precaution against misinterpretation. But even in instances where the risk of misinterpretation is unlikely, loss of productivity as a result of contamination is a serious issue.

As the author of this white paper notes, determining if an artifact on a slide is a contaminant or patient tissue can require minutes, or even hours, of extra time in analysis. With a critical shortage of pathologists, productivity is an issue that laboratories cannot ignore. In a survey done in 2007 by Washington G-2 Reports, more than 75% of anatomic pathology groups reported difficulties in recruiting pathologists. With the average age of pathologists over 50, this shortage will grow more critical as baby boomers retire.

With the advent of new technology that promises to effectively protect against contamination via staining baths, this may be the time for laboratories to address this issue. If reduced cross-contamination can be realized, laboratories have the opportunity to not only improve patient safety, but also to improve a critical laboratory issue – pathologist productivity.
1. Background

Contamination of anatomic pathology slides is a recognized event in laboratory medicine. A 1996 College of American Pathologists retrospective review\(^1\) found contaminant tissue fragments, ranging from a few cells to entire extraneous tissue sections, on approximately 3% of anatomic pathology slides. Other studies report slide contamination rates of up to 25%.\(^1,2\)

Most contaminants, often called “floaters” by laboratory staff, are easily recognized as such. However, depending on the tissue being evaluated and the clinical circumstances, contamination can be problematic for the pathologist. While misinterpretation due to slide contamination is rare, a contaminated slide usually requires additional time to fully evaluate. Preparation and examination of deeper sections of the tissue is often necessary, and more time is usually needed for careful consideration of the diagnostic possibilities suggested by the extraneous tissue. Occasionally, expensive molecular approaches, such as DNA analysis, are used to objectively resolve questions of contamination.\(^3,4\)

Accurate recognition of a slide contaminant requires the presence of histologic features that differentiate a floater from the tissue under examination. As a result, contamination of patient samples by tissue of a similar type may pose a relatively higher risk for misinterpretation. Floaters consisting of neoplastic (e.g., malignant) tissue fragments are considered particularly dangerous because they may resemble pathologic processes that naturally occur in many tissue types. For example, adenocarcinomas of the lung, colon, bladder, breast, salivary gland, and other organs strongly resemble...
one another. Squamous cell carcinomas can occur in many organs and are often identical, regardless of the tissue of origin.

On the other hand, some pathologic processes are characterized by normal-appearing tissue developing in an inappropriate location. In this clinical setting, a benign contaminant, that might otherwise be recognized as such, could lead to misdiagnosis of a pathological lesion. For example, intestinal metaplasia of the esophagus can resemble normal intestinal mucosa, but represents a dangerous premalignant condition in the appropriate clinical setting.

Acute awareness of these risks arose through a study, currently in progress, of contaminants found in H&E linear stainer baths. The purpose of the study is to measure the frequency of slide contamination in multiple different laboratories and to determine the number and types of contaminants found in the stainer baths. While final data is not yet available, preliminary data indicates that contamination by tissues commonly believed to cause diagnostic difficulty (e.g., fragments of neoplasm) does occur, but also that other problematic tissue types are seen with regularity. The frequent finding of intestinal and squamous epithelium in the staining baths may indicate higher risks for misinterpretation of esophageal biopsies and endocervical curettage specimens than pathologists currently recognize.
2. Sources of Contamination of Patient Slides

Contamination of blank slides sent through the stainer occurred at a rate of up to 25%, depending on the time of day when the slides were processed.

The first opportunity for contamination occurs during gross examination and dissection. Contaminants introduced on the grossing table or during the process of embedding tissue in paraffin can be very difficult to discern from patient tissue because the contaminant consistently appears on every cut section. In this setting, the pathologist must rely on histologic and clinical context in order to resolve the problem. When necessary, molecular techniques can be used to establish identity.

Many laboratory personnel believe the water bath to be a common source of contamination. However, in a recent study of the histology laboratory at the Cleveland Clinic, 195 liters of water from 13 different baths were examined, yet only one floater was identified. In the same experiment, staining baths from a linear H&E stainer were found to contain a total of 696 tissue fragments. Contamination of blank slides sent through the stainer occurred at a rate of up to 25%, depending on the time of day when the slides were processed. These data indicate that fragmentation of tissues within the staining baths of linear stainers is likely to be a significant, and perhaps under-recognized, source of slide contamination.
3. Specimen Types with Additional Risk

While all tissue types can present a risk of incorrect diagnosis, analysis of contaminants found in staining baths highlights the risks associated with certain tissue types. This paper addresses three specimen types that are considered high-risk for misdiagnosis due to cross-contamination: esophageal biopsies, endocervical curettage specimens, and lymph nodes biopsied for metastatic malignancy. Misinterpretation may include either false-positive diagnoses (not recognizing a floater as contaminant tissue), or false-negative diagnoses (erroneously attributing a small focus of true disease to contamination).

**Esophageal biopsies**

Barrett’s esophagus, a precursor lesion to adenocarcinoma of the esophagus, is characterized by intestinal metaplasia, in which esophageal tissue takes on a histological appearance similar to that of normal intestine. It can be diagnosed with very focal histologic changes. The finding of intestinal-type epithelium in the esophagus is required for a diagnosis.

A diagnosis of Barrett’s esophagus carries serious clinical and economic implications for the patient, including the need for frequent endoscopic surveillance and the psychological impact of a premalignant diagnosis.

Contamination of an esophageal biopsy by a very small fragment of normal tissue from the small intestine or colon may lead to a false-positive diagnosis of Barrett’s esophagus, especially in a specimen consisting of multiple fragmented biopsies.
Contamination of an esophageal biopsy by a very small fragment of normal tissue from the small intestine or colon may lead to a false-positive diagnosis of Barrett’s esophagus, especially in a specimen consisting of multiple fragmented biopsies. Contamination by a fragment of atypical or “dysplastic” intestinal epithelium (e.g., from a colon polyp) might cause misinterpretation of the lesion as Barrett’s esophagus with “dysplasia”; a diagnosis with even more serious clinical consequences. Conversely, in laboratories with a high incidence of slide contamination, a pathologist might conceivably render a false-negative diagnosis by ascribing a small focus of true intestinal metaplasia to contamination by a stray fragment of intestinal epithelium.

Normal-appearing small and large intestinal epithelium contaminants are a common finding in linear H&E staining baths. The presence of even a small slide contaminant on a biopsy from the lower esophagus, especially in a patient with a history of reflux and a suggestive endoscopic evaluation, could lead to a misdiagnosis of Barrett’s esophagus. For this reason, esophageal biopsies are considered a high-risk specimen type.

![Image](image.png)

**Fig.1:** Biopsy from the lower esophagus showing a fragment of gastric cardia-type mucosa with scattered oxytic glands. A single detached fragment of intestinal-type epithelium is also present. In this case, a diagnosis of Barrett’s esophagus was considered, but given the lack of continuity with the associated columnar mucosa, contamination is a distinct possibility.
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Fig. 2: Biopsy from the lower esophagus showing glandular mucosa and two detached fragments of intestinal-type epithelium. The presence of goblet cells in this specimen meets histologic criteria for a diagnosis of Barrett’s esophagus, but the lack of continuity among the three pieces raises the possibility of contamination.

Fig. 3: Biopsy from the lower esophagus showing a fragment of gastric cardia-type mucosa and several detached fragments of columnar mucosa with goblet cells. Adjacent tissue levels did not show the fragments of intestinal-type epithelium. A diagnosis of Barrett’s esophagus was initially rendered on the basis of this field, but later reconsidered given the possibility of slide contamination. The patient will be undergoing increased surveillance due to the appearance of this slide.
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Fig. 4: Biopsy from a patient with abnormal mucosa in the lower esophagus. This section shows hyperplastic and reactive-appearing columnar mucosa, as well as a detached fragment of intestinal-type epithelium. This appears to meet criteria for a diagnosis of Barrett’s esophagus, but the small piece of intestinal epithelium was not present on deeper sections, suggesting the possibility of slide contamination from a fragment of small intestinal mucosa.

Fig. 5: Biopsy from the lower esophagus showing columnar mucosa and a single detached fragment of goblet cell-containing epithelium resembling a colonic crypt (upper left). This meets histologic criteria for a diagnosis of Barrett’s esophagus, but the slide shown in Fig. 6 raises doubts.
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Fig. 6 shows the same area as Fig. 5, but on a deeper section. The intestinal epithelium is absent on this level, raising concern for slide contamination.

Curettage specimens

The nature of sampling during curettage, especially endocervical curettage, makes detection of slide contamination difficult. The tissue is fragmented, and the mucosal architecture is often disrupted. Cross-contamination by atypical “dysplastic” or malignant squamous epithelium could potentially lead to a false-positive diagnosis. Since fragments of squamous epithelium are among the most common contaminants observed in linear H&E stainer baths, the risk of misinterpretation due to slide contamination in endocervical curettage specimens is considered high. For similar reasons, other curettage specimens (e.g., endometrial) may be subject to a higher risk of interpretation error due to cross-contamination, by either atypical squamous or glandular tissue fragment.
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Fig. 7: Section of an endocervical curettage specimen showing a single fragment of dysplastic squamous epithelium, which is not in direct association with the remaining tissue. The degenerated and fragmented appearance strongly resembles the changes observed in stain bath contaminants. The atypical epithelium was not present on deeper levels, raising concern for slide contamination.

Fig. 8: Section of an endocervical curettage specimen showing a single fragment of dysplastic squamous mucosa. This was the only abnormality observed in the sample, and given the lack of association with the surrounding tissue fragments and mucus, slide contamination was considered a possibility.
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Slide contaminants are reported to consist of neoplastic tissue in up to 12.7% of reported cases.¹

Fig.9: Section of an endocervical curettage specimen showing a fragment of dysplastic squamous epithelium and cervical mucus. This was the only abnormality seen in the entire specimen. The atypical fragment was not present on adjacent tissue levels. While the atypical fragment does seem to be in close association with the mucus, the absence of other supportive findings raised concern for slide contamination.

Lymph node biopsy for metastatic malignancy

Another area considered to have an increased risk for misinterpretation due to tissue cross-contamination is lymph node evaluation for metastatic malignancy, including sentinel node biopsies. Slide contaminants are reported to consist of neoplastic tissue in up to 12.7% of reported cases.¹ Contamination of lymph node sections by even a small fragment of malignant tissue could lead to a false-positive diagnosis. While the lack of a stromal reaction may alert the pathologist to the possibility of cross contamination, micrometastases often do not invoke such a reaction, and so the risk of misinterpreting a slide with a small floater may be even higher than it would be with a large contaminant.
4.

Elevated Risks for Specialty Laboratories

For labs that specialize in one of the anatomic pathology disciplines (e.g., dermatopathology, gastrointestinal pathology, urologic pathology, or hematopathology), the risk of misinterpretation due to cross-contamination is considered particularly high. In these practice settings, the preponderance of specimens are of a similar type. As a result, contaminants are likely to appear microscopically similar to patient tissue under examination. One of the most important factors enabling a pathologist to recognize contamination is the morphologic contrast between dissimilar tissue types, which most floaters happen to be by sheer chance. For example, a piece of prostate tissue on the same slide as a woman’s breast biopsy specimen is likely to be recognized as contamination. On the other hand, in a practice that exclusively evaluates prostate biopsies, a stray fragment of prostate is less likely to be identified as extraneous. The ability to discern contamination from test tissue is inherently limited when most specimens processed in a given laboratory are from the same organ system.
Cost of Contamination

Most pathologists consider floaters to be more of a nuisance than a serious risk for misdiagnosis. However, in a College of American Pathologists Q-Probes study of extraneous tissue on surgical pathology slides, examination of additional tissue sections was required for 12.2% of contaminated slides, and the degree of diagnostic difficulty was graded as “severe” by the examining pathologist in 0.4% of cases. In 0.6% of cases, pathologists could not definitively distinguish extraneous tissue contaminants from patient tissue with microscopy alone.

While this data suggests that true diagnostic dilemmas due to slide contaminants are relatively rare, cross-contamination can be troublesome.

Even when the pathologist is able to confirm that a suspected floater is really a contaminant, the additional analytical time and need for additional tissue sections decreases productivity of the entire lab.

Even when the pathologist is able to confirm that a suspected floater is really a contaminant, the additional analytical time and need for additional tissue sections decreases productivity of the entire lab. For cases in which microscopy alone cannot distinguish contamination from patient tissue, use of molecular identity testing or even re-biopsy may be needed. Molecular testing is time-consuming and of substantial cost, while re-biopsy exposes the patient to additional procedure-related risk and is not always possible.

In addition, the credibility and reputation of the laboratory is at risk, if only from the reporting delay needed to fully evaluate a case with slide contamination.
A single misdiagnosis could be catastrophic to the patient and laboratory alike. For the patient, exposure to unnecessary treatment or a delay in the start of lifesaving therapy are both possible outcomes of a misdiagnosis. For the laboratory, such outcomes also pose the risk of expensive lawsuits.

Considering the cost of evaluating contaminated slides and the risk of misdiagnosis, protecting against slide contamination in the anatomic pathology laboratory deserves serious consideration. Productivity issues alone may be sufficient to warrant consideration of technology that can protect against cross-contamination, especially if the institution is already considering investing in new staining equipment.
6.

Protecting Against Contamination

To reduce the productivity loss and risk of misinterpretation due to slide contamination, labs have a variety of options. If contamination at the grossing station or embedding table is an issue, adherence to a clean technique and maintenance of a tidy workspace is the best solution. If staff habits have grown lax, retraining and close supervision may be needed.

Contamination during the staining process is a more complex issue. Because the nature of the process — shared baths combined with the agitation that occurs in a linear stainer — create an environment conducive to cross-contamination of slides, process changes can help protect the integrity of patient samples. Studies have shown that higher slide volumes correlate with increased frequency of slide contamination (more contamination in the late afternoon). As a result, more frequent reagent changes may likely reduce the incidence of cross contamination, though the possibility of such contamination still exists.

The best protection against contamination in the staining process may be elimination of shared staining baths. Ventana Medical Systems, Inc., is the first company to produce a commercial staining system that does not use shared baths. Its instrument, called Symphony instrument system, differs significantly from other technology in that a staining module discretely stains individual slides, using fresh reagent for each slide. In comparison to batch staining, where reagents can be contaminated or degrade over many uses, Symphony equipment provides consistent, reproducible results.
While no system can claim to eliminate cross-contamination, the Symphony H&E system has eliminated a significant source of contamination, i.e., the staining baths.

The Symphony also allows technologists to fine-tune staining protocols for enhanced results for particular tissue types. The individual staining also allows technologists to easily alter the staining protocol to improve contrast on slides that are particularly challenging. Also, because the reagents are packaged in cassettes, solutions can be changed without process interruption. Up to 25 user-defined protocols can be used simultaneously, and an infinite number of protocols can be saved.

The Symphony universal slide tray holds up to 20 individual slides that can be stacked or “nested” for pathologist review. The Symphony “Slide Detect” ID module can read multiple barcode formats to allow slides to be tracked within the process. Multiple slide trays can be processed simultaneously, including drying, de-paraffinization, staining and applying cover slips.

For a complete description of the technology used, please refer to the Ventana web site: http://www.ventana.com/product/page?view=symphony.
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7.

Conclusion

Tissue fragmentation within the staining baths of linear H&E stainers is common and may lead to more slide contamination than is currently recognized. While misdiagnosis due to slide contamination is probably rare, and most slide contamination is relatively easy to recognize, certain specimen types may be at an increased risk for misinterpretation in the setting of contamination.

Among tissue types, esophageal biopsies for intestinal metaplasia, curettage specimens for squamous dysplasia, and lymph node biopsies for metastatic malignancy are all considered high-risk specimens. Identifying contaminated slides may be more difficult in a specialized laboratory due to the similar morphologic appearance of floaters and patient samples. Possible negative outcomes include patient harm and the increased time/cost of evaluating a contaminated slide. Because of these risks, pathologists, technologists and administrators should consider focusing quality efforts on reducing the rate of contamination in the laboratory. Attention should be given to batch linear H&E stainers as a possible under-recognized source of slide contamination. If contamination via staining baths is detected and considered problematic, solutions might include increasing the frequency of reagent changes or utilization of the Ventana Symphony individual slide staining technology.
References


Appendices
A-1
About John Carpenter, MD

John B. Carpenter, M.D., is a pathologist at Pacific Pathology Partners and Harrison Medical Center in Silverdale and Bremerton, Washington. He has an interest in gastrointestinal pathology, immunohistochemistry, and laboratory management.

Dr. Carpenter attended medical school at the University of Washington School of Medicine, and completed a residency in anatomic and clinical pathology at the University of Arizona. He was a Robert E. Petras Fellow in gastrointestinal pathology at the Ameripath Institute of Gastrointestinal and Liver Pathology in Cleveland, Ohio, and has authored or co-authored 19 research papers and abstracts.

Dr. Carpenter was compensated by VENTANA® Medical Systems for his participation in preparing this report.
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About Ventana Medical Systems, Inc.

Ventana Medical Systems, Inc. (“VMSI”) develops, manufactures, and markets instrument/reagent systems that automate tissue preparation and slide staining in clinical histology and drug discovery laboratories worldwide. The Company’s clinical systems are important tools used in the diagnosis and treatment of cancer and infectious diseases. VMSI’s drug discovery systems are used to accelerate the discovery of new drug targets and evaluate the safety of new drug compounds. In addition, the Company offers premier workflow solutions designed to improve laboratory efficiency, providing safeguards to enhance the quality of healthcare. Ventana Medical Systems, Inc. is a wholly-owned member of the Roche Group. For more information on Ventana Medical Systems, Inc. visit www.ventana.com.

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About DARK Daily

“DARK Daily is a concise e-news/management briefing on timely topics in clinical laboratory and anatomic pathology group management. It is a solution to the dilemma facing anyone in the laboratory profession. New developments, new technology, and changing healthcare trends make it imperative to stay informed to be successful. At the same time, the Internet, cell phones, blackberries, laptop computers and wireless devices are overwhelming any one individual’s ability to absorb this crushing Tsunami of data.

DARK Daily is a quick-to-read, easy-to-understand alert on some key development in laboratory medicine and laboratory management. It has no counterpart in the lab world. Why? Because it is produced and written by the experts at THE DARK REPORT and The Dark Intelligence Group, who know your world, understand your needs and provide you with concise, processed intelligence on only those topics that are most important to you!

You will find DARK Daily to also be an exceptionally valuable resource in laboratory and pathology management. Some of the lab industry’s keest minds and most effective experts will be offering their knowledge, their insights and their recommendations on winning strategies and management methods. Many of these experts are unknown to most lab directors. As has proven true with THE DARK REPORT for more than a decade, DARK Daily will be your invaluable—and unmatched—resource, giving you access to the knowledge and experience of these accomplished lab industry professionals.
About The Dark Intelligence Group, Inc. and THE DARK REPORT

The Dark Intelligence Group, Inc., is a unique intelligence service, dedicated to providing high-level business, management and market trend analysis to laboratory CEOs, COOs, CFOs, pathologists and senior-level lab industry executives. Membership is highly-prized by the lab industry’s leaders and early adopters. It allows them to share innovations and new knowledge in a confidential, non-competitive manner. This gives them first access to new knowledge, along with the expertise they can tap to keep their laboratory or pathology organization at the razor’s edge of top performance.

It offers qualified lab executives, pathologists and industry vendors a rich store of knowledge, expertise and resources that are unavailable elsewhere. Since its founding in 1996, The Dark Intelligence Group and THE DARK REPORT have played in instrumental roles in supporting the success of some of the nation’s best-performing, most profitable laboratory organizations.

The Dark Intelligence Group (TDIG) is headquartered in Austin, Texas. This location makes it very accessible for any laboratory organization seeking input, insight and support in developing their business operations, creating effective business strategies and crafting effective sales and marketing programs that consistently generate new volumes of specimens and increasing new profits. The Dark Intelligence Group, Inc. owns and operates two Web sites in the TDIG Website network:


http://www.DarkDaily.com
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About the Executive War College on Laboratory and Pathology Management

Every spring since 1996, the lab industry’s best and brightest gather at the Executive War College on Laboratory and Pathology Management to learn, to share and to network. Many consider it to be the premier source of innovation and excellence in laboratory and pathology management.

Each year, a carefully selected line-up of laboratory leaders and innovators tell the story of how their laboratories are solving problems, tackling the toughest challenges in lab medicine and seizing opportunities to improve clinical care and boost financial performance. The Executive War College is the place to get practical advice and solutions for the toughest lab management challenges. A unique case study format brings participants face-to-face with their most successful peers. They tell, first hand, how their laboratory solved intractable problems and successfully used new technology.

Many lab management secrets are shared, along with specific “what-not-to-do’s” gained from hard-won experience! It’s not pie-in-the-sky theory, but useful knowledge that can be put to use in any lab. The Executive War College offers superlative networking, with lab administrators and pathologists attending from countries as far away as the United Kingdom, Germany, Brazil and Australia. It makes the Executive War College a melting pot for all the best ideas, new lab technologies and management strategies now reshaping the laboratory industry. It’s also become a recruiting ground used by headhunters and major lab organizations.

In the United Kingdom, The Dark Intelligence Group and the Association of Clinical Biochemists (ACB) have co-produced a meeting every February since 2003. Known at Frontiers in Laboratory Medicine (FiLM), it attracts laboratory leaders and innovators in the United Kingdom. Also featuring a case study format, this meeting pioneered the international laboratory side-by-side case study, where a North American laboratory and a United Kingdom laboratory prepare a comparison of best practices and an operational assessment of their two organizations.
In September 2005, a laboratory management meeting called Executive Edge was conducted in Toronto, Ontario, Canada, by The Dark Intelligence Group and QSE Consulting. It provided pathologists and lab directors in Canada with a customized meeting devoted to the strategic and operational issues of laboratory management in Canada.
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About Karen Branz

Karen Lynn Branz is the owner of Branz Communications, which provides professional writing and editing services with a concentration in health care journalism and technology topics. Her career has included newspaper and magazine work as well as stints in marketing for health care systems and as communications director for a health care quality improvement organization. In addition to her work with The Dark Intelligence Group, her clientele has included Health Leaders Media, the Texas Hospital Association, Ventana Medical Systems, The American Heart Association, The Nielsen Healthcare Group, 3M, Motorola, Seton Healthcare System, Scott & White, and other health and technology organizations.

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