Cross-contamination of pathology specimens are a significant concern in the anatomic pathology and histology laboratories. Errors in diagnosis have severe medico-legal consequences and implications for both the patient and his or her team of clinicians, including the pathologist. Investigation of potential sources of cross-contamination, and preventative action are of utmost importance in reducing diagnostic error.

Taking fresh tissue samples from the circuitous route of the clinical visit to the pathologist’s microscope requires many steps, most of which include hands-on manipulation. Many of the defects in specimen processing can be attributed to the large number of steps in taking tissue from the fresh state to the point of diagnosis. In the setting of a busy and often understaffed pathology laboratory, each of the manual steps in tissue processing is a potential point of error.

Few “gold standards” exist in anatomic pathology. Analysis of morphology is subjective, and based primarily on an observer’s practical experience. Although some observers have more experience or are more competent than others, all observers are human and are subject to the problems of human limitations. While this source of error is unavoidable, and part of the inherent anatomic pathology paradigm, cross-contamination or carry-over of specimens can be minimized by awareness and corrective action on the part of the pathologists’ assistant and histotechnologist.

Most laboratories have implemented a variety of quality controls to reduce the risk of carry-over and “floaters.” There may be a policy to avoid serial accessioning and grossing of similar specimens. This may reduce the risk of misidentifying sequential specimens, but can also prevent carry-over artifacts between like specimens from using contaminated forceps or scalpels.

Carryover can occur at every step, including adherence to instruments during prosection, but can also occur inside the tissue processor and at many steps in the histology process. A comprehensive study of slide contamination was undertaken by Platt, et al, at the Cleveland Clinic, with the focus of their study taking place in the histology lab. This study used standard formalin fixation in 10% neutral buffered formalin and paraffin embedding of tissues on all samples. Tissue processing was performed using standard techniques on traditional processor, and typical microtome and water bath setups were used. Hand-labeled slides were stained using a semiautomated linear stainer, racked with a maximum of 20 slides per rack, dipped sequentially into each bath for the appropriate amount of time, and coverslipped on a separate automated coverslipper. Fluids that were collected and analyzed for the study were processed on the ThinPrep machine. For the assessment of contaminants, slides were stained in a continuous workflow discrete slide stainer (Symphony).

The investigation was aimed at determining whether contaminants in the stainer baths could be carried over to other slides during the process of staining, and whether the contaminants in the
water baths could be picked up on slides and be the source for tissue floaters. There were three experiments performed to assess the phenomenon.\textsuperscript{4}

The first experiment assessed slides that had tissue on them for the presence of tissue floaters picked up during the staining process. Ten source tissues of different types, including colon cancer, endometrial curettings, lung parenchyma, bone marrow with bone spicules, fibrous tissue, breast cancer, fibrous breast tissue, skin, thyroid, and gastrointestinal stromal tumors were used. The specimens were cut into different-sized fragments: 2 mm, 4 mm, and 6 mm in diameter, and were embedded into 3 paraffin blocks, respectively. Using standard laboratory protocols, the histotechnologist cut forty slides from each block. Twenty slides were stained in the linear stainer and 20 stained in the Symphony stainer. The slides were then assessed for external tissue contaminants and floaters, discohesion of the known tissue fragments, and for movement of these discohesive fragments from one area of the slide to another.\textsuperscript{4}

Platt, et al., then assessed the possibility of cross-contamination occurring in the staining baths as they passed through the linear stainer. At the end of an average work day, they sent 40 routine histology blank slides through the stainer, without any other tissue bearing slides in the machine. They also sent 40 slides through the Symphony stainer for comparison, and 200 charged slides were sent through the stainer in alternating positions with slides that had routine tissue on them at regular intervals throughout the course of an average work day. Then all of the slides were assessed microscopically for the presence of any tissue contaminants, defined as fragments that were more than 2 cells in size and contained at least one nucleus, excluding single keratinocytes.\textsuperscript{4}

In tissue sections containing known source tissues, displacement of fragments of the tissue across the slide, especially in friable tissue types, like the colon cancer specimens, were frequently noted. Platt, et al, surmised that this was due to discohesion or lifting of the tissue fragments during staining. The linear stainer had significantly more of these displaced tissue fragments than the Symphony stainer, 44\% vs. 22\%. Two of the slides from the linear stainer (3\% of the slides stained) had foreign tissue fragment floaters, or contaminants that did not match the type of tissues contained in the known source tissue block. None of the slides stained on the Symphony had foreign contaminants.\textsuperscript{4}

Of the 40 blank slides run through the linear stainer and the 40 blank slides run through the Symphony stainer, none picked up any contaminants. However, when the researchers alternated the blank slides with tissue sections, 16 of the 200 blank charged slides harbored 1-3 tissue floaters. It was noted that the contaminants were small, with an average size of 13.9 cells (range, 4–50 cells).\textsuperscript{4}

They found that contaminants are present in the water bath by the microtome in very low concentrations. Of the thirteen water baths analyzed, representing the equivalent of 13 different histotechnologists cutting 15-20 blocks per hour for an 8 hour shift, only one tissue fragment (lymph node tissue) was identified. The microscopic examination was remarkable for the presence of some acellular contaminants in the water baths including keratin, fragments of paraffin, and minute specks of India ink. It was determined that tissue sections found in the
water bath are maintained within a thin sheet of paraffin, and fragmentation of tissue that can cause floaters is less likely at this point in processing.

However, the potential for tissue contamination during staining is higher, most likely because the tissue is deparaffinized during the first steps of an H&E stain. As the slides are agitated, the newly deparaffinized tissue can fragment and dis cohesive pieces can break free from the slide. This was especially prevalent in tissues that were friable, fragmented or naturally less adherent to slides, such as the colon cancer specimens. The contaminants were most abundant in the early baths, particularly the first set of xylenes and first set of alcohols (Figure 1).4

The percentage of diagnostic slides with tissue floaters or contaminants was estimated at around 3%. Up to 30% of these floaters consisted of abnormal or frankly malignant tissues.4 These figures suggest that roughly 1 out of every 100 slides examined could potentially contain malignant tissue that leads to a false diagnosis. Many times these contaminants can be resolved at the histologic level, and are easy to confirm when the tissue contaminant stems from a different organ. Molecular analysis, specifically DNA fingerprinting, can resolve most potential tissue floaters provided the error is caught by the pathologist examining the slide.

There are other investigators and researchers that have looked into this phenomenon as well, with similar results. A study by Layfield, et al, reviewed quality assurance (QA) records for floaters in slides and additionally examined 1,000 current slides for floaters. In the 521,661 slides they reviewed, 65 floaters were detected: 28 were present in the tissue block, and 37 were found only in a single level. In the 1,000 prospectively examined slides, 12 floaters were detected; 9 were only in a single section. They concluded that floaters remain a source of diagnostic error and occur in 0.01% to 1.2% of slides.5

How does the laboratory resolve issues of cross contamination? The answer is not clear-cut, and several different methods can be utilized with similar results. It has been suggested that there are three crucial steps in the proper investigation of contaminants, starting with a clinical and laboratory correlation.6

The first step in resolving any suspected cross contamination should start with a review of the clinical history, procedure or operative note, and communication with the clinician. Information about the lesion biopsied, and the clinician’s impression at the time of the procedure should be reviewed. Next, review the gross description and any notes that were made by the pathologists' assistant, resident, or grossing technologist. The number of tissue fragments described should be compared to the number present in the block or on the slide. Discussion with the individuals involved in grossing, embedding, and cutting of the tissue may also offer some insight. Inquiry regarding any deviations from normal practice can offer insight in isolating the issue. Next, investigate what other specimens were grossed that day, or even around the same time. Was there a large and friable tumor that may have been the source of a contaminant? As was mentioned earlier, one can occasionally rule out contamination on histologic grounds if the contaminant does not match the organ that was biopsied.6

In the event that the floater cannot be readily ruled out as a contaminant, an immunohistochemical stain work-up should be implemented as the next step. This can help
resolve more challenging cases, especially when the tissue is present in the block, and is not just an extraneous piece found on the slide. One shortfall of immunohistochemistry, however, is that many malignancies have overlapping staining patterns, further challenging the investigation. If this scenario is applicable in your case, it is prudent to conserve tissue for molecular analysis. Avoiding an exhaustive immunohistochemical workup is wise if molecular testing is indicated.6

As a final resort, DNA fingerprinting should be explored.6 A complex procedure, DNA fingerprinting first involves microdissection of the tissues to be examined. Following careful dissection, polymerase chain reaction (PCR) should be performed for predetermined polymorphic microsatellite markers that are diverse enough to allow discrimination between separate specimen sources. Finally, allele size and content should be analyzed by electrophoresis, and the genotypes compared for identity.7

The following case study illustrates a similar scenario. A 33-year-old woman presented with a fracture of the left humerus. Her history was notable for a large solitary bone cyst of the humerus first resected and repaired in 1992. She continued to sustain multiple fractures at this site, and elected to have many surgeries to correct this problem. In 2002, following one of these surgeries, bone fragments of her left humerus were submitted for routine surgical pathology.8

The majority of the specimen consisted of bone and fibrous tissue with scant marrow, showing otherwise normal hematopoietic elements. There was, however, an unexpected finding. A small (1.0 x 1.0 mm) tissue fragment with a sheet-like array of small lymphocytes was noted. Immunohistochemistry confirmed these as CD20 and CD5 expressing B cells, with focal expression of CD23 and no detectable CD10. The pathologist diagnosed this as a “single, small, detached fragment of soft tissue with apparent involvement by a low-grade, CD5-positive B-cell neoplasm of uncertain clinical significance.” The pathologist also reported that he “could not entirely exclude the possibility that this soft tissue fragment was actually derived from a different specimen and was artifically embedded in . . . this case.”8

The patient was asymptomatic and chose not to pursue treatment for this diagnosis, but was compliant with annual follow up to include CBC’s and physical examinations. Five years later, she moved to a different city, and began seeing a new hematologist-oncologist for annual follow-up of her presumed lymphoma. Her annual blood counts and examinations were continually negative for conditions concerning lymphoma. This prompted the oncologist to initiate a review of the original pathology material.8

The original diagnosis of devitalized bone and fibrous tissue from 1992 was confirmed. The bone from 2002 was also reviewed, and the tissue fragment with the atypical small B-cell population was identified (Figure 2). Analysis comparing DNA extracted from both the suspicious fragment of tissue from the biopsy in question and the patient’s peripheral blood leukocytes was suggested.8

Ten unstained recut slides from the 2002 paraffin block were received for further study. The suspicious tissue fragment was identified and dissected away from the surrounding uninvolved bone tissue. The DNA was recovered and used for short tandem repeat (STR) analysis. DNA
from the patient’s peripheral blood was used for comparison. DNA from the B-cell lymphoma tissue fragment and DNA from the patient’s peripheral blood were distinguished using PCR amplification for 9 highly polymorphic independently segregating STR loci and 1 gender-specific locus. Amplification products were subjected to capillary electrophoresis and the unique DNA fingerprint patterns were analyzed.8

The genotype for 9 polymorphic loci and a gender-specific locus observed from this DNA was able to be distinguished from the genotype pattern of the peripheral blood DNA isolated from the patient. The most convincing evidence is from the sex chromosomes. The patient is a woman and homozygous for a single peak at 104 base pairs in length corresponding to DNA amplified from the X chromosome. In contrast, DNA extracted from the B-cell lymphoma tissue yields 2 peaks corresponding to 104- and 110-bp fragments consistent with a DNA pattern obtained from a man. These fragments represent amplified DNA from the X and Y chromosomes, respectively. In addition to Y chromosome, which would otherwise be enough evidence to show cross-contamination in this case, specific regions of DNA from the B-cell lymphoma tissue generated PCR fragments not present in the patient at the majority of the loci studied (Figure 3). Together, these data indicate that the B-cell lymphoma tissue within the block was not derived from the patient.8

This patient, who chose to forgo immediate treatment and instead opted for regular monitoring of her condition, was spared the physical trauma of treatment. I cannot speculate on the emotional toll or angst that a presumed diagnosis of lymphoma may have had, but I imagine it made an impact. This study is a good reminder to all laboratory professionals that our actions and work make an impact in people’s lives, every day. As pathologists’ assistants, there are a number of steps we can take to help minimize potential errors. Paying careful attention to maintaining a clean workspace is the first step. Properly scrubbing and sanitizing your tools between specimens, cleaning cutting boards and replacing chucks frequently, and changing scalpel blades and gloves often can prevent direct carry over from our workspace, instruments or hands. Due diligence when dealing with friable, fragmented or naturally non-adherent tissue, primarily by containing the specimen in HistoWrap, or other containment system for friable materials, can help reduce the incidence of carryover during processing. Encouraging and helping the histotechnologists and laboratory assistants to filter or change the baths in the slide stainer regularly, in particular the 2nd-5th xylenes, the 2nd 100% alcohol, and both 95% alcohol baths found in the beginning of the staining sequence, will also help reduce the incidence of floaters due to the staining process. Working together as a team of laboratory professionals, we can help reduce the incidence of diagnostic error.
FIGURE 1: Graph of number of contaminating fragments from the stainer baths when the entire bath was evaluated. The x-axis has the stain bath identity and the y-axis has the overall number of contaminants on the ThinPrep slide that was prepared from the contents.\textsuperscript{4}
FIGURE 2. Low-power microscopy of the original slide from 2002 bone reamings (H&E, 40). The tissue fragment in the upper right-hand corner (circled in green) is the lymphoid fragment of interest. 2
FIGURE 3. Electropherogram following amplification of genomic DNA by PCR for 9 STR loci and a gender-specific marker. The y axis illustrates the peak height or the amount of fluorescent PCR product detected. The x axis corresponds to the size of the amplification products as noted by arrows in base pairs (bp). Red peaks correspond to size standards at 139, 150, 150, 200, 250, 300, 340, and 350 bp fragments, respectively. (A) Results from peripheral blood. (B) Results from B-cell lymphoma tissue.
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