

TECHNICAL NOTE

Pathology/Biology

A method for the development of cranial fracture histology slides

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Funding information

National Institute of Justice, Grant/Award Number: 2017-DN-BX-0166

Abstract

Cranial vault fractures are of medicolegal interest as they have long-term impacts to someone's health and may contribute to an individual's death. The ability to distinguish antemortem from perimortem fractures and to assess the age of the injury is increasingly dependent on histology. Despite the increasing role of histology in assessing the microanatomy of osseous fractures, there are no methods currently available which account for the nuances and difficulties in creating high-quality histologic slides of cranial vault fractures that allow visualization of cellular features associated with healing bone. The authors present a modified method specific to slide development of human cranial vault fractures derived from the trial-and-error process of creating 730 such slides over a 3-year period which are suitable for the evaluation of the tissues, cells, and nuclei involved in fracture healing. This method adapts and troubleshoots typical histological procedures including sample excision, fixation, decalcification, dehydrating, clearing, embedding, microtomy, and staining, and introduces new procedures including preprocessing photography and cassette placement. By implementing these modifications, the number of poor-quality slides that required a new section to be sent to the histology laboratory was greatly reduced. Proactively implementing this new method into cranial fracture histologic slide development significantly reduces the number of slide rejections due to common issues like folding, chatter, or insufficient staining, saving both time and financial resources for forensic practitioners, researchers, and histotechnologists.

KEYWORDS

bone histology, cranial fractures, decalcified bone, forensic anthropology, forensic pathology, fracture dating, fracture histology, histology method, histotechnology

Highlights

- Presents a method for producing histologic slides of cranial fractures from fixation to staining.
- Provides troubleshooting for common microtomy and staining complications.
- Tested protocols for reducing the number of slide rejections.
- Useful for forensic and medical specialists for producing quality cranial fracture histology slides.

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1 | INTRODUCTION

Fractures of the cranial vault represent serious injuries that are often the focus of examination in medicolegal death investigations due to their serious and potentially long-lasting impacts on an individual's health and contributions to their death. A key question asked by forensic pathologists and anthropologists is the age of a cranial fracture relative to the death event. These estimates of fracture age contribute to accurately characterizing the cause and manner of an individual's death. Broad assessments of antemortem or perimortem injuries can be made using visual or radiologic examination of osseous healing; however, cases of multiple antemortem injuries require a finer-detailed assessment to date injuries and determine if the fractures are consistent with a single traumatic instance or multiple episodes of trauma. In recent decades, microscopic assessments of fractures using histology have shown greater promise in both detecting and dating skeletal injuries. Kleinman and colleagues [1] utilized histology in concert with specimen radiography to increase detection of fractures in infants during postmortem examination, while Klotzbach et al [2] found histology both increased fracture detection and was able to elucidate the early microscopic signs of healing not apparent using radiography. Cappella et al [3] and Delabarde et al [4] both found histology was superior to radiography and micro CT for fracture age estimation. A 2019 study by Naqvi et al [5] presents an algorithm to age postcranial fractures in infants based on histological features.

It is apparent from the prevailing research that histology is the gold standard for detection of tissues and cells involved in the healing trajectory. Furthermore, it is the identification of these tissues and cells on high-quality histological slides that will ultimately allow the evaluation and accurate estimation of time since fracture. The development of histologic slides suitable for microscopic analyses of osseous and adjacent tissues is critical for this endeavor. Numerous processes including fixation, decalcification, dehydration, clearing, embedding, microtomy, staining, and mounting are used to transform excised specimens of wet bone into stained histologic slides. Each of these steps must be carefully undertaken to produce usable and informative specimens; however, problems such as incomplete decalcification, folding artifacts, and ineffective staining may arise, which necessitate alterations to the established protocols. While protocols for decalcified bone histology are available [6, 7], they often apply to smaller samples (e.g. bone biopsy) or nonhuman bones and there are no resources specific to osseous fracture histology of human cranial bone despite the importance of histologically assessing head injuries in medicolegal death investigations.

This technical report presents a method for developing samples of cranial vault fractures into histologic slides. The method was developed over 3 years as part of a research study funded by the National Institute of Justice (2017-DN-BX to investigate the histomorphology of healing cranial fractures. Many of the early samples required recuts due to folding, chatter, artifact, or poor staining. As

the study progressed, the rejection rate decreased dramatically due to modifications in the histology protocols. As such, this method was derived from the creation of 730 histologic slides of calvarial fractures from injuries sampled at autopsy from infant (0–3 years), juvenile (3–16 years), and adult (16+ years) decedents. Samples included linear, comminuted, depressed, diastatic, and hinge fractures to the cranial vault, as well as surgically induced defects (e.g., craniotomy/craniectomy/cranioplasty, burr hole, ventriculoperitoneal shunt). The post-traumatic survival time of the injuries ranged from acute to 42 years. The purpose of this report is to provide forensic practitioners and researchers with a successful methodology for developing histological slides from cranial vault fractures and ways to troubleshoot common problems that may arise. This method follows standard histology procedures for bone samples with important modifications to improve the resulting slides for the evaluation of fracture histomorphology. Table 1 provides a comparison between standard histologic procedures, the modified method presented, and the resultant outcomes. This method can serve as a baseline for the development of fracture histology techniques in collaboration with histotechnologists providing forensic pathology services. The sections below describe detailed methodology for processing human cranial bone fractures from excision to staining and methodological solutions to common problems that are often encountered with bone histology.

2 | MATERIALS AND METHODS

The materials and solutions required to carry out the components of this method prior to histological processing include the following:

- Reciprocating autopsy bone saw, rotary tool with blade attachment, or neurosurgical saw
- Plastic specimen jar(s)
- 10% neutral buffered formalin
- Decalcification solution of choice—5% nitric acid or 10% ethylenediaminetetraacetic acid (EDTA) are recommended
- Orbital shaker (optional)
- Scalpel(s)
- Tissue embedding cassette(s)
- Slide/cassette markers for labeling (optional)
- Biopsy sponge(s) (optional)
- 70% ethanol
- Digital camera equipment
- Radiography equipment

The chemical solutions and equipment needed for histological processing onward are readily available in laboratories which have the capacity to perform bone histology. Any relevant product numbers and equipment specifications used by our histology laboratory are included within the protocols for reference but are not necessary for the execution of this procedure.

TABLE 1 Comparison of standard bone histology methods and associated issues to the proposed modifications in this report and the resulting quality improvements

	Standard histologic practice	Issues	Fracture modifications	Quality improvements
Sample excision and fixation ^a	10% neutral buffered formalin (NBF) for shorter times.	High possibility of incomplete fixation causing decomposition of cells and tissues.	10% NBF for 14 days for infant and 30 days for juvenile/adult samples	Complete fixation. Excellent microscopic morphology.
Decalcification ^a	Hydrochloric acid (HCl) or other harsh decalcifying chemical for 1–3 days. No radiographs to determine endpoint.	High possibility of incomplete decalcification which makes microtomy difficult or over-decalcification which destroys microscopic components.	Juveniles/adults: 5% nitric. Infants: 10% EDTA. 6–10 days with agitation & frequent reagent refresh. X-ray to monitor decalcification.	Accurate determination of decalcification endpoint. Microscopic components maintained with excellent morphology.
Cassette placement	Sample placed in cassette unsupported. No photographs taken.	Loss of fracture orientation. Damage/loss from mechanical agitation within cassette.	Cut samples to size of cassette or use sponges to brace sample. Photograph sample in profile.	Fracture orientation maintained or reconstructed from photographs. Reduced damage to fractures.
Dehydrating, clearing	Standard short processing programs.	Insufficient penetration of bone samples. Difficult to cut tissue. Poor section quality.	Extend processing program times for complete penetration of processing reagents.	More easily sectioned, complete, high-quality sections.
Embedding and microtomy	Sample placement flat, any orientation	Knife edge can catch fracture edges and damage the sample.	Sample oriented with consideration of fracture site in relation to cutting direction.	Sample protected from damage during cutting. Reduced chatter and folding.
Staining	Routine hematoxylin and eosin (H&E) stain.	Standard microscopic assessment.	Routine H&E stain, trichrome, AB/OG, and pentachrome	Additional microscopic structures identified allowing detailed assessment of fracture healing.

^aIndicates the most crucial steps for obtaining high quality slides for fracture histomorphology.

2.1 | Sample excision and fixation

Upon identification of the calvarial fracture of interest, photograph the fracture site in situ (Figure 1A) and excise the fracture from the decedent utilizing an autopsy bone saw, rotary tool with blade attachment, or neurosurgical saw. Radiograph the specimen to determine the kVp and mAs setting at which the entire sample is radiopaque. This will set the baseline for determining when the specimen is completely decalcified. We recommend radiograph settings of 40–48 kVp and 0.9 to 1.8 mAs for infant and other nondiploic bone and 50 kVp and 2.5 mAs for adult and juvenile samples. Next, photograph the excised specimen from both the ectocranial (Figure 1B) and endocranial (Figure 1C) views and place the specimen into 10% neutral buffered formalin at a ratio of 15–20 times the volume of the specimen in a plastic specimen jar to fix for a minimum of 14 days for infant samples and 30 days for juveniles and adults. If the sample will also be examined macroscopically by a forensic anthropologist or practitioner, it may be carefully divided to avoid disrupting the fracture or an additional sample of the fracture should be taken and not subjected to fixation. After fixation, rinse the sample under running water for at least 1 h to prevent any formalin artifact or negative impacts on staining on the final slides and to avoid the creation of toxic carcinogens when combined with decalcification agents.

Complete fixation of the entire sample is necessary to ensure tissues do not decompose during the decalcification process and prevent tissue distortion by dehydrating and clearing agents [8]. A general rule is that a sample with cortical bone will fix at a rate of approximately

2 mm per 24 h [9] and samples that are 3–4 mm cubes of compact bone should fix for at least 1 week [8]. Fixation times may be reduced by trimming samples to a smaller size; however, this may cause the disruption of the fracture site. Fixation rates may be increased with the use of heat, but this must be applied with caution as heat will also increase the rate of decomposition [8]. If available, the use of vacuum can increase the fixation rate by approximately 2.5 times [8].

2.2 | Decalcification

Following fixation and rinsing, immerse the sample in the chosen decalcification agent approximately 20 times the volume of the sample at room temperature on an orbital shaker set to 110–125 revolutions per minute. Decalcification agents used over the course of this method's development include 7% hydrochloric acid (HCl), 5% nitric acid, or 10% ethylenediaminetetraacetic acid (EDTA). We recommend, however, that HCl not be utilized for decalcifying cranial bone due to the poor quality of slides developed from specimens decalcified in HCl. Furthermore, EDTA provides the best preservation of microscopic features but at the cost of long processing times in bone samples greater than 3 mm. Since infant bones are typically less than 3 mm, EDTA can be used without sacrificing expediency. For juvenile or adult bones, however, nitric acid is a better option with faster decalcification that also results in high-quality histologic slides. For further discussion regarding decalcification agents and their impact on bone histomorphology, please see Cornelison et al [10]. Throughout

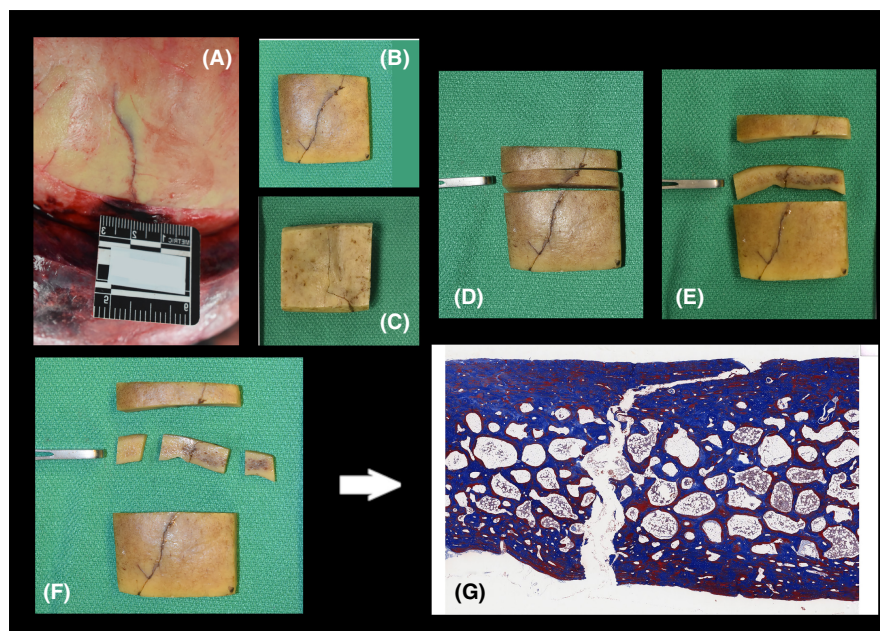


FIGURE 1 Recommended preprocessing photographic series: (A) fracture in situ, (B) excised fracture sample from an ectocranial view, (C) excised fracture sample from an endocranial view, (D) thick section of the fracture sample from the ectocranial view with forceps indicating the section used for slide development, (E) cross-sectional view of the thick section of the fracture sample, (F) trimmed fracture sample in cross-section. The resulting histologic slide: (G) photomicrograph of the fracture sample stained with Masson's trichrome (scanned with Aperio CS2 digital slide scanner). Note how the fracture margin morphology seen in the slide is also visible in (E,F) as is the curvature of the inner table. The preprocessing photography can help to orient the slide and identify the outer and inner tables. [Color figure can be viewed at wileyonlinelibrary.com]

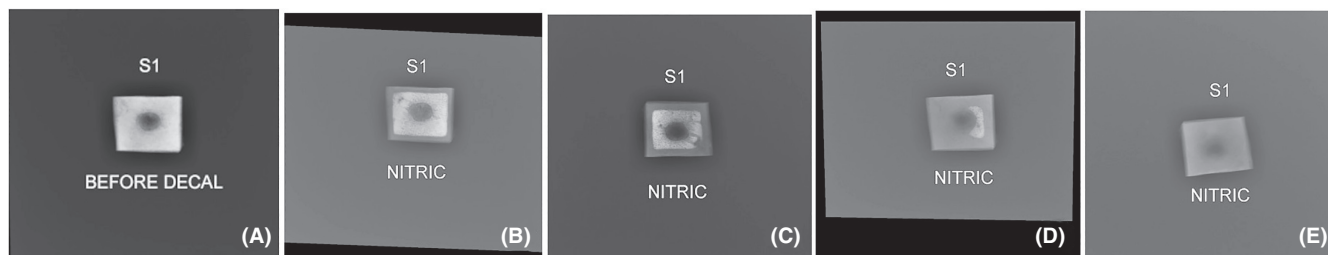


FIGURE 2 Radiographic decalcification assessment of a burr hole defect immersed in 5% nitric acid (A) before decalcification, (B) 5 days immersed, (C) 7 days immersed, (D) 10 days immersed, and (E) complete decalcification at 14 days. A setting of 50 kVp and 2.5 mAs was used for each radiograph.

immersion decalcification, the sample should be monitored radiographically to establish the decalcification endpoint using the setting previously identified for the sample (see *Sample Excision and Fixation*). Juvenile and adult samples immersed in nitric acid should be radiographed every 1 to 2 days while those decalcifying in EDTA should be checked at intervals no greater than 10 days to ensure the specimens are not degraded due to over-decalcification. Infant samples immersed in EDTA should be radiographed every 2 days to evaluate decalcification. Figure 2 shows the radiographic progression of decalcification. The chosen decalcification agent should be replaced each time the sample is radiographed to ensure the decalcification process continues. Typically, infant samples will decalcify in EDTA in approximately 6–10 days and juvenile/adult samples will decalcify in nitric acid in 6–10 days. Upon reaching consistent radiolucency across the specimen, rinse the sample under running water for at least 2 h to halt the decalcification process. Place the samples in 70% ethanol if the sample will not be immediately trimmed and placed in a cassette after rinsing.

The use of an orbital shaker is not necessary, but the agitation may increase the rate of decalcification as will frequent changes to the decalcification solution [11, 12]. Increasing the temperature at which decalcification occurs can also reduce decalcification time but can lead to poorer histomorphology due to the maceration of tissues [11, 12]. Smith [13] suggests 25°C for acid decalcification while Kapila and colleagues [11] found 40°C to hasten decalcification but at the cost of poor bone marrow cell details, osteocyte retraction, and folds. If radiography is not available to ascertain the decalcification endpoint, chemical tests may be used to detect presence of calcium precipitate. Alternatively, the weight loss method pioneered by Lillie and colleagues [9] and tested by many others [14, 15] determines decalcification is complete when the bone sample reaches a constant weight after consistent decrease during the decalcification process. Alternatively, samples may be tested using a needle or scalpel away from the fracture site, but this is generally considered unreliable, potentially damaging to the sample, and may miss undecalcified bone islands that will make microtomy impossible [11, 16].

2.3 | Cassette placement

Use a scalpel to cut a thick section from the middle of the fracture sample, as shown in Figure 1(D). Obtaining a section from the middle

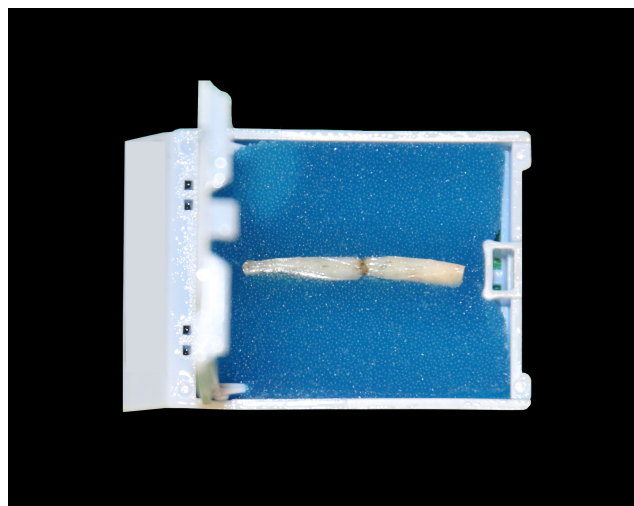


FIGURE 3 Fractured infant bone braced in a biopsy sponge within a cassette. Bracing with the sponge prevents sample movement during processing and ensures the fracture margins remain approximated. [Color figure can be viewed at wileyonlinelibrary.com]

of the specimen will remove any irregular edges which may be present due to the autopsy saw cut. When possible, trim the sample to the approximate length and thickness of the cassette to stabilize the specimen throughout processing. Photograph both the ectocranial and cross-sectional views of the trimmed section to highlight the diploic bone and fracture margins (Figure 1D–F). Preprocessing photography is not a requisite element for histologic slide development; however, photographic documentation of the fracture sample will aid in identifying the specific location from which the section was taken, fracture margins in cases of extensive healing, and the outer and inner tables of the cranium on the final slides (Figure 1G).

Once a thick section has been trimmed and photographed, place it into a processing cassette in profile. If the cranial fracture section is thin, such as infant and temporal vault specimens, the sample should be braced within the cassette to approximate the fracture margins and prevent the sample from moving during processing. Brace the sample using a biopsy sponge cut to the dimensions of the cassette with a slit incised in the center of the sponge. Nestle the thin fracture sample into the slit as shown in Figure 3. Once the fracture is in place, affix the cassette lid and ensure the cassette is labeled with the appropriate case or laboratory number, using a solvent-resistant

cassette marker if necessary. Place the fracture tissue in the labeled cassette into 70% ethanol solution while awaiting tissue processing. The remnants of the larger sample may also be placed in 70% ethanol solution for storage. In our office, the preceding steps of Sample Excision and Fixation, Decalcification, and Cassette Placement are performed by a forensic practitioner or staff member to ensure care and to reduce efforts required by the histology laboratory. The following steps of Dehydrating, Clearing, and Embedding, Microtomy, and Staining are completed by histotechnologists.

2.4 | Dehydrating, clearing, and embedding

Tissue processing, encompassing specimen dehydrating and clearing, follows standard histology procedures and can be performed using an automated platform or via traditional bench methods. Once processing is complete, the histology technician should evaluate the tissue sample for orientation and placement into the block mold. This is highly dependent on the size and type of bone specimen, as well as the fracture site(s). Consideration should be given to how the blade surface will interact with the sample, balancing the overall angle of both the surrounding bone and the fracture surfaces. Minimize the parallel orientation of the microtome blade and fracture margin by embedding the sample at an angle to the blade (Figure 4). Remove the fracture sample from the cassette (and sponge if used) and transfer it to the embedding mold in the same position, taking care not to disrupt the fracture site. Embed the specimen in paraffin wax at 60°C and affix it to a cassette. Cool the sample to solidify the paraffin using either a cold plate or ice block in preparation for sectioning. Our histology laboratory uses a Sakura Tissue-Tek VIP E300 on a 30-h program outlined in Table 2 for tissue processing, EpreDia Type 6 Paraffin 8336 wax for embedding, and an EpreDia HistoStar embedding station with cold module for sample cooling.

2.5 | Microtomy

To improve the final section quality, face the paraffin block at a thinner setting and hydrate the block during the microtomy process. Section the chilled and well-hydrated paraffin block at 4–6 μm using a rotary microtome. Float the tissue sections on a heated water bath to remove wrinkles and retrieve the sections on charged glass slides. This method does not use any adhesive or additive on the slide or in

the water bath. Individual laboratories should assess the impact of additives and adhesives on staining before use. Dry the slides either at room temperature overnight or in a drying oven for a minimum of 1 h at 60°C prior to staining. An EpreDia HM 355S Automated Rotary Microtome is used by our histology laboratory to section the samples, a Boekel 145701 Standard Lighted Tissue Flotation Bath is used to float sections, and a Thermo Scientific Slide Oven—High Capacity Section Dryer B3120202 for drying the slides.

If problems are encountered during microtomy resulting in poor section quality (e.g. chatter, knife marks, folding, wrinkling) (Figure 5), first evaluate the angle of the sample and the angle of the fracture in relation to the blade. The block should be oriented to reduce the horizontal contact between the blade and the embedded sample (Figure 4). Reducing the cutting speed or changing the orientation of the block in the microtome chuck can also help. If necessary, the paraffin block can be melted down and the tissue re-embedded at a different orientation or flipped over to attempt cutting on the naïve surface. Any rotation should be documented to maintain an accurate understanding of the fracture position.

Excessive knife marks, unavoidable chatter, or the blade pulling the tissue from the block indicate the tissue is not completely decalcified. This may occur even though radiography indicates the sample is fully decalcified. To troubleshoot, place the cut face of the trimmed paraffin block face down in a dish containing a small amount of decalcification solution for approximately 10–20 min, then re-chill the block and attempt to section the specimen again. If this does not help, another sample from the excised fracture specimen should be cut to ensure complete decalcification. The original fracture sample should be removed from the 70% ethanol holding solution, rinsed for 1 h under running water, and re-immersed in the corresponding decalcification agent for no more than 2 days for nitric acid and 5 days for EDTA. To assess the decalcification progress, radiograph the specimen using a lower setting than previously used. Once the sample appears thoroughly decalcified, rinse the sample for 2 h, and resume sample development from “Cassette Placement” onward.

2.6 | Staining

After the fracture tissues are mounted and dried, the tissues should be stained according to standard protocols for the stains being used. For visualizing cranial fracture histomorphology, we recommend a series of four stains: Masson's trichrome [17], hematoxylin and eosin

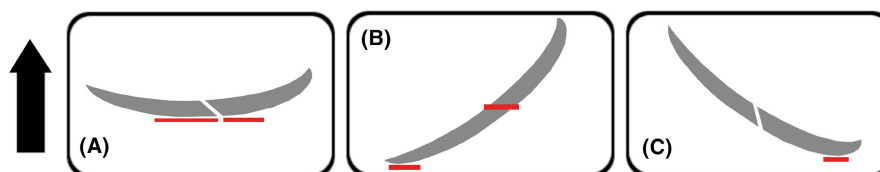


FIGURE 4 Orientation of fracture sample relative to microtome blade. Arrow indicates direction of blade travel with potential poor blade interaction sites marked in red. (A) Orienting the sample parallel to the blade is not recommended. (B) Overall bone is oriented at an angle, but the fracture edge could be problematic. (C) A balanced angle of orientation for the overall bone and fracture edges. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Recommended automated processing program for cranial fracture tissues

Cycle	Solution	Concentration	Duration (h:min)	Set temp.	P/V	Agit
1	Fixative—Neutral Buffered Formalin (Thermo/Epredia 5701)	10%	0:00	N/A	On	On
2	Fixative—Neutral Buffered Formalin	10%	0:05	N/A	On	On
3	Dehydrant (Thermo/Epredia 6215)	70%	0:45	N/A	On	On
4	Dehydrant	95%	0:45	N/A	On	On
5	Dehydrant	95%	1:30	N/A	On	On
6	Dehydrant	100%	3:00	N/A	On	On
7	Dehydrant	100%	3:00	N/A	On	On
8	Dehydrant	100%	4:00	N/A	On	On
9	Xylene (Thermo/Epredia 6601)	100%	4:00	N/A	On	On
10	Xylene	100%	4:00	N/A	On	On
11	Paraffin (Thermo/Epredia Type 6 Paraffin 8336)	N/A	2:00	60°C	On	On
12	Paraffin	N/A	3:00	60°C	On	On
13	Paraffin	N/A	4:00	60°C	On	On
14	Paraffin	N/A	0:00	60°C	Off	Off

Note: The program is derived from the Standard Operating Procedures for the Research Histology Laboratory at Western Michigan University Homer Stryker M.D. School of Medicine.

Abbreviations: P/V, pressure/vacuum; Agit, agitation.



FIGURE 5 Common artifacts resulting from microtome sectioning. (A) H&E slide with folding along the fracture margins. (B) Trichrome slide with knife marks indicated by oblique striations. (C) AB/OG slide of a fracture in advanced healing which shows extensive wrinkling of the sample along inferior margin. [Color figure can be viewed at wileyonlinelibrary.com]

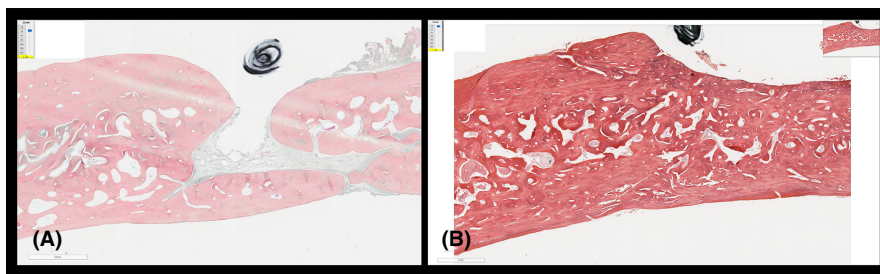


FIGURE 6 The variable results of AB/OG staining. (A) A washed-out AB/OG slide due to old or exhausted staining reagents. (B) A properly stained AB/OG slide with fresh reagents. [Color figure can be viewed at wileyonlinelibrary.com]

(H&E) [18], Russell-Movat pentachrome [19] and alcian blue hematoxylin with an orange G counterstain (AB/OG) [20]. This creates a series of at least four stained slides of each fracture sample.

Over-differentiation may cause washed-out nuclei on H&E slides and washed-out elastic fibers on pentachrome slides. If this occurs, re-section the specimen and closely monitor the differentiation step for H&E and pentachrome under a microscope. In the case of AB/OG, the intensity of the reagents will noticeably decrease over time

and during periods of high-volume slide development. If AB/OG slides appear washed-out (Figure 6), re-section the specimen and stain with refreshed AB/OG reagents. Once the slides are stained, apply a mounting medium to the slide and overlay a glass coverslip. An automated coverslipping system may also be used to complete the slide set(s). While Masson's trichrome, H&E, and Russell-Movat pentachrome are standard stains in histology laboratories, the AB/OG stain is less common, and this method used VitroView Alcian

Blue Hematoxylin/Orange G Stain Kit VB-3002. The mounting medium used was ClearVue Mountant, EpreDia 4211 and the automated coverslipping system was a Thermo/EpreDia ClearVue Automatic Coverslipper with Slide Basket Transfer System.

3 | CONCLUSION

Protocols specific to the development of histologic slides from cranial fractures and defects are notably absent throughout the literature despite histology being considered the most valuable modality to elucidate the details of fracture age. While the methods outlined herein follow standard histological procedures for hard tissues, our experience developing cranial fractures/defects into histologic slides resulted in new modifications, protocols, and tips for troubleshooting which are easily implemented in forensic and histology laboratories. As histology laboratories are often not accustomed to processing larger human bone samples, it is important for forensic practitioners to advocate for modifications to standard procedures to ensure high-quality results to inform their investigation. The new developments reported herein improved outcomes for histologic slide development of cranial vault fractures. Integration of these guidelines can assist forensic practitioners, researchers, and histotechnologists in optimizing the quality of histologic slides for the assessment of fracture histomorphology, reducing the number of slide rejections saving time and resources, and preventing the unnecessary destruction of samples.

ACKNOWLEDGMENTS

The authors acknowledge Erica Christensen, M.S., of Western Michigan University Homer Stryker M.D School of Medicine, Department of Pathology, for her assistance with sample preparation. The authors thank the two reviewers for their thoughtful evaluations which helped to improve this manuscript.

FUNDING INFORMATION

This work was supported by the National Institute of Justice [award number 2017-DN-BX-0166]. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the U.S. Department of Justice.

CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

REFERENCES

- Kleinman PK, Marks SC, Richmond JM, Blackburne BD. Inflicted skeletal injury: a postmortem radiologic-histopathologic study in 31 infants. *Am J Roentgenol*. 1995;165(3):647-50. <https://doi.org/10.2214/ajr.165.3.7645487>
- Klotzbach H, Delling G, Richter E, Sperhake J, Püschel K. Post-mortem diagnosis and age estimation of infants' fractures. *Int J Leg Med*. 2003;117(2):82-9. <https://doi.org/10.1007/s00414-002-0338-3>
- Cappella A, de Boer HH, Cammilli P, De Angelis D, Messina C, Sconfienza LM, et al. Histologic and radiological analysis on bone fractures: estimation of posttraumatic survival time in skeletal trauma. *Forensic Sci Int*. 2019;302:109909. <https://doi.org/10.1016/j.forsciint.2019.109909>
- Delabarde T, Reynolds M, Decourcelle M, Pascaretti-Grizon F, Ludes B. Skull fractures in forensic putrefied/skeletonised cases: the challenge of estimating the post-traumatic interval. *Morphologie*. 2020;104(344):27-37. <https://doi.org/10.1016/j.morpho.2020.01.002>
- Naqvi A, Raynor E, Freemont AJ. Histological ageing of fractures in infants: a practical algorithm for assessing infants suspected of accidental or non-accidental injury. *Histopathology*. 2019;75:74-80. <https://doi.org/10.1111/his.13850>
- Carson F, Hladik C. *Histotechnology: a self-instructional text*. 3rd ed. Chicago, IL: American Society for Clinical Pathology Press; 2009. p. 1-76.
- An YH, Martin KL. *Handbook of histology methods for bone and cartilage*. Totowa, NJ: Humana Press; 2003. p. 97-286.
- Jenkins LL, Burg KJL. Tissue harvesting and fixation. In: An Y, Martin KL, editors. *Handbook of histology methods for bone and cartilage*. Totowa, NJ: Humana Press; 2003. p. 143-57.
- Lillie RD, Laskey A, Greco J, Burtner HJ, Jones P. Decalcification of bone in relation to staining and phosphatase technics. *Am J Clin Pathol*. 1951;21(8):711-22. <https://doi.org/10.1093/ajcp/21.8.711>
- Cornelison JB, Isaac CV, Devota CJ, Billian J, Brown TT, DeJong JL, et al. A comparison of three decalcification agents for assessments of cranial fracture histomorphology. *J Forensic Sci*. 2022;67(3):1157-66. <https://doi.org/10.1111/1556-4029.14990>
- Kapila SN, Srikant N, Boaz K, Pandya JA, Yinti SR. Driving the mineral out faster: simple modifications of the decalcification technique. *J Clin Diagnostic Res*. 2015;9(9):ZC93-7. <https://doi.org/10.7860/JCDR/2015/14641.6569>
- Sterchi DL. Bone. In: Suvarna SK, Layton C, Bancroft J, editors. *Bancroft's theory and practice of histological techniques*. 11th ed. Amsterdam, Netherlands: Elsevier; 2019. p. 280-305. <https://doi.org/10.1016/C2015-0-00143-5>
- Smith A. The use of frozen sections in oral histology. I. *J Med Lab Technol*. 1962;1:26-38.
- Mawhinney WH, Richardson E, Malcolm AJ. Control of rapid nitric acid decalcification. *J Clin Pathol*. 1984;37(12):1409-13. <https://doi.org/10.1136/jcp.37.12.1409>
- Sanderson C, Radley K, Mayton L. Ethylenediaminetetraacetic acid in ammonium hydroxide for reducing decalcification time. *Biotech Histochem*. 1995;70(1):12-8. <https://doi.org/10.3109/10520299509108310>
- Skinner RA, Hickmon SG, Lumpkin CK, Aronson J, Nicholas RW. Decalcified bone: twenty years of successful specimen management. *J Histotechnol*. 1997;20(3):267-77. <https://doi.org/10.1179/his.1997.20.3.267>
- Carson F, Hladik C. Masson trichrome stain. *Histotechnology: a self-instructional text*. Chicago, IL: American Society for Clinical Pathology Press; 2009. p. 162-4.
- Carson F, Hladik C. H&E staining. *Histotechnology: a self-instructional text*. Chicago, IL: American Society for Clinical Pathology Press; 2009. p. 114-5.
- Carson F, Hladik C. Russel modification of the Movat pentachrome stain. *Histotechnology: a self-instructional text*. Chicago, IL: American Society for Clinical Pathology Press; 2009. p. 172-3.
- VitroVivo Biotech. *VitroView™ Alcian Blue Hematoxylin/Orange G stain kit SKU#: VB-3002*. 2017. [cited 2021 Nov 15]. Available from: <https://vitrovivo.com/wordpress/wp-content/uploads/2018/01/VB-3002-user-manual-1.pdf>

How to cite this article: Isaac CV, Cornelison JB, Devota CJ, Bailey K, Langworthy J. A method for the development of cranial fracture histology slides. *J Forensic Sci*. 2022;67:2040-2047. <https://doi.org/10.1111/1556-4029.15093>