Protocol

Multimodal Characterization of Postmortem Pink Teeth in Indonesian Forensic Practice: Protocol for a Retrospective Laboratory Case Series

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Abstract

Background: Postmortem pink teeth are an uncommon finding resulting from color changes within dental tissues. They have been reported in several death contexts, including drowning, but neither their presence nor their intensity is specific to a cause of death. Inconsistent photographic, radiological, and histological methods have limited comparisons across studies.

Objective: This study aims to describe a reproducible multimodal workflow for postmortem pink teeth and assess the feasibility, measurement reliability, and exploratory associations of protocol-specified photographic scores, cone-beam computed tomography (CBCT) structural measurements, and histological distribution scores.

Methods: This retrospective laboratory case series will use existing specimens and records from Indonesian forensic practice. Candidate teeth will undergo outcome-blind eligibility assessment to establish applicable ethics coverage, provenance, medicolegal custody, anonymous decedent linkage, and technical evaluability. Photography will be scored using the 3-level Score of Pink Teeth Color and Score of Pink Teeth Region scales. CBCT will be used to quantify dentin thickness and the pulp-to-tooth area ratio after long-axis reorientation; CBCT gray values will not be interpreted as tissue density. Hematoxylin-eosin sections will be scored according to the farthest dentinal zone containing recognizable erythrocytes or blood-compatible pigmented material, without claiming chemical identification of hemoglobin. Two blinded raters will evaluate each modality. Analyses will emphasize descriptive estimates, weighted kappa, intraclass correlation coefficients, and exploratory Spearman correlations, with bootstrap 95% CIs. Multiple teeth from the same decedent will be handled by decedent-level clustering when linkage is available. No diagnostic accuracy or cause-of-death prediction analysis will be performed.

Results: The working catalog currently includes 25 candidate teeth with existing photographic and CBCT records and available histopathological source materials. Research ethics approval was renewed (004123/KEP Ladokgi R.E. Martadinata/2026) and is valid from July 29, 2026, to July 29, 2027. The renewed approval describes the study as involving 25 postmortem pink-teeth samples. All candidate teeth will undergo outcome-blind eligibility assessment based on ethics coverage, provenance, medicolegal custody, decedent linkage, and technical evaluability. Protocol-locked remeasurement and blinded histological rereview will be completed only for eligible specimens before the final dataset is frozen. Reliability assessment and integrated analysis will be performed using the frozen analytic dataset. Candidate specimens were collected between 2023 and 2025. Protocol-locked assessments are scheduled for November to December 2026, followed by analysis through January 2027. Preparation of the subsequent results manuscript is planned for January 2027; its publication date has not been determined.

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Conclusions: The protocol prioritizes traceable acquisition, conservative biological interpretation, and reliability over diagnostic claims. It is intended to standardize the documentation of a rare forensic finding and identify methods suitable for subsequent prospective validation.

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KEYWORDS

pink teeth; forensic odontology; postmortem change; cone-beam computed tomography; histopathology; dental photography; case series; protocol

Introduction

Background

Postmortem pink teeth describe a pink-to-red discoloration of teeth observed after death. Proposed mechanisms include intrapulpal blood accumulation, erythrocyte breakdown, and the movement of blood-derived pigments through dentinal tubules [1-9]. The finding has been documented in cases involving drowning, asphyxia, intoxication, trauma, and other circumstances; however, systematic and case-based evidence does not support its use as a stand-alone marker of drowning or any other cause of death [4,5,10-17].

Drowning remains an important preventable mortality problem internationally and in Indonesia [18,19], while dental methods contribute to standardized disaster victim identification [20,21]. This public health and operational context does not increase the diagnostic specificity of postmortem pink teeth.

Observed color may depend on tooth type, pulp vitality before death, dentin thickness, humidity, decomposition, and the interval and conditions between death, recovery, imaging, and tissue processing [2-5,10,11,13,14,16]. These sources of variation make isolated visual descriptions difficult to compare. A multimodal workflow can link standardized appearance to tooth structure and microscopic distribution, but each modality must retain its evidentiary limits. Photography records surface color, cone-beam computed tomography (CBCT) records structure rather than chemical pigment, and routine hematoxylin-eosin staining records morphology rather than molecular identity.

Recent reviews have reinforced the need for cautious interpretation of postmortem pink teeth. Contemporary evidence indicates substantial heterogeneity in reported circumstances, proposed mechanisms, photographic documentation, histological assessment, and the interpretation of associated postmortem findings [10-12]. This heterogeneity limits direct comparison across studies and supports a methodological shift away from cause-of-death attribution toward standardized characterization, measurement reliability, and explicit reporting of evidentiary limitations. A multimodal protocol that preserves the distinct contributions and limitations of photography, CBCT, and histopathology may therefore provide a more reproducible framework for subsequent validation studies.

The practical use case is therefore documentation and quality assurance, not diagnosis. A reproducible workflow may improve case comparison, make uncertainty explicit in forensic reports, and generate reliability estimates for subsequent prospective validation. This protocol follows a data-existing approach, because photographic and CBCT records already exist, while eligibility adjudication, protocol-locked remeasurement, histological review, and integrated analysis remain incomplete.

Objectives

The primary objective is to estimate interrater reliability for the protocol-specified photographic, CBCT, and histological measurements. Secondary objectives are to describe the distributions of these measurements, quantify technical completeness, and explore cross-modal associations. Cause-of-death information, when independently and lawfully available, will be summarized only as case context. The study will not estimate sensitivity, specificity, likelihood ratios, diagnostic thresholds, or posterior probabilities.

Methods

Study Design and Setting

This retrospective laboratory case series concerns postmortem pink teeth handled within Indonesian medicolegal practice and studied at the Faculty of Dentistry, *Universitas Padjadjaran*. This revised protocol is version 2.3, dated September 1, 2026, and supersedes version 2.2, dated July 19, 2026. Changes made after review of the existing records, including the updated ethics authorization, are documented in the amendment Table 1 and are not presented as prospectively prespecified. Table 1 identifies changes made after review of the existing dissertation records. These changes represent transparent retrospective amendments rather than claims of original prespecification.

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Table 1. Retrospective protocol amendments and rationale.

Earlier concepts	Current protocol decision	Reason
Exploratory cross-sectional predictor study	Retrospective descriptive laboratory case series	Selection based on pink-teeth status does not support diagnostic inference.
Original ethics documentation referred to 20 specimens, while the working catalog contained 25 candidate teeth	Research ethics approval was renewed (004123/KEP Ladokgi R.E. Martadinata/2026) and describes a study scope involving 25 pink-teeth samples. Each candidate will undergo specimen-level verification of ethics coverage, provenance, medicolegal custody, decedent linkage, and technical evaluability before inclusion in the analytic cohort.	To align the protocol with the current written ethics authorization while retaining specimen-level verification of ethics coverage, provenance, medicolegal custody, decedent linkage, and technical evaluability
Delta E color analysis	Score of Pink Teeth Color and Score of Pink Teeth Region ordinal scores only	ABFO[a] number 2 is a geometric scale, not a calibrated color target.
CBCT[b] density or erythrocyte spread	Dentin thickness and pulp-to-tooth area ratio	Uncalibrated CBCT gray values do not provide quantitative measures of tissue density.
H&E[c]-based hemoglobin identification	Morphological zone score	H&E staining alone does not chemically identify hemoglobin.
Generic histological description	Approved SOP-PT-HISTO-001 Revision 1.0 and a specimen-level evidence register	The approved standard operating procedure standardizes histopathological processing, documentation, quality control, specimen-level traceability, and protocol-locked histological assessment while distinguishing historical source materials from protocol-locked activities.
Diagnostic accuracy and Bayesian prediction	Descriptive, reliability, and exploratory association estimates	No representative pink-negative comparator or valid diagnostic denominator is available.

[a]ABFO: American Board of Forensic Odontology.

[b]CBCT: cone-beam computed tomography.

[c]H&E: hematoxylin-eosin.

Ethical Considerations

The study initially received ethics approval from the research ethics committee of Ladokgi RE Martadinata (004108/KEP Ladokgi R.E. Martadinata/2025). The ethical authorization was subsequently renewed (004123/KEP Ladokgi R.E. Martadinata/2026) on July 29, 2026, and is valid from July 29, 2026, to July 29, 2027. The renewed approval describes a study scope involving 25 pink-teeth samples for CBCT and histopathological examination. Each candidate tooth will nevertheless require specimen-level verification of ethics coverage, provenance, medicolegal custody, decedent linkage, and technical evaluability before inclusion in the analytic cohort.

Candidates that do not satisfy these requirements will be excluded, or the affected modality will be classified as nonevaluable, when appropriate. The renewed approval is not interpreted as retrospective authorization for historical procedures performed before its effective period; therefore, historical source materials will be distinguished from protocol-locked activities conducted under the current protocol.

All records used for analysis will be coded. Names, police case numbers, precise recovery locations, and facial or other directly identifying images will not be included in the analytic dataset. Consent, waiver, or other applicable authorization status and the legal basis for secondary research use will be documented for each included decedent based on the applicable written ethics and medicolegal records. No waiver or individual authorization will be assumed in the absence of supporting documentation.

Candidate Specimens and Final Analytic Cohort

The working catalog contains 25 candidate teeth with visible postmortem pink discoloration. The candidate catalog is a convenience series and is not a consecutive sample of all deaths or all extracted teeth. Each candidate tooth will be linked to an anonymous decedent identifier before analysis so that the numbers of teeth and unique decedents can be reported separately. No predetermined number of candidate teeth will be excluded solely on the basis of specimen count. Final cohort membership will be determined by documentary eligibility and modality-specific technical evaluability.

A tooth will be included if it is a permanent tooth with visible pink-to-red discoloration, has verifiable research authorization, documented provenance and medicolegal custody, can be linked to an anonymous decedent identifier, and has at least 1 technically evaluable study modality. A tooth will be excluded if the required authorization, provenance, custody, or decedent linkage cannot be verified, or if damage prevents identification of the anatomical landmarks required for the study. When an otherwise eligible tooth has an inadequate source photograph, Digital Imaging and Communications in Medicine (DICOM) series, histological slide, or processing record for a specific modality, that modality will be classified as nonevaluable rather than automatically excluding the tooth from the entire study. No missing modality result will be imputed.

The eligibility assessment will be conducted without reference to modality-specific scientific results. For each candidate code, the eligibility record will document the eligibility status,

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documentary basis, reviewer, and decision date. Only candidates meeting the applicable documentary eligibility requirements will proceed to protocol-locked remeasurement, blinded histological rereview, reliability assessment, and integrated analysis. Candidates excluded during the eligibility assessment will not enter the final analytic cohort.

Study Flow and Assessment Framework

The tooth is the measurement unit, and the decedent is the clustering unit when more than 1 tooth originates from the same individual. Because this retrospective laboratory case series uses postmortem dental specimens and existing records rather than involving the enrollment of living participants, participant flow is represented as specimen flow. The study begins with the working catalog of 25 candidate teeth, followed by outcome-blind documentary eligibility assessment, anonymous decedent linkage, modality-specific technical evaluability assessment, protocol-locked photographic and CBCT remeasurement, blinded histological assessment, quality control resolution, final dataset freeze, reliability analysis, and exploratory cross-modal analysis. Candidates that do not satisfy the required documentary eligibility criteria will not enter the final analytic cohort, whereas an otherwise eligible candidate may remain in the cohort when a specific modality is classified as nonevaluable.

No participant-facing intervention is delivered in this retrospective laboratory case series; therefore, intervention delivery and participant acceptability are not applicable. Data are derived from existing photographic, DICOM, histopathological, and documentary source materials together with protocol-locked measurements to be performed under the current protocol. Feasibility is defined as the proportion of eligible candidates yielding a technically evaluable result for each modality, with explicit numerators, denominators, and reasons for nonevaluability. Outcome measures and planned analyses are specified in the Protocol-Specified Outcomes and Statistical Analysis sections.

Data Management and Confidentiality

The linkage file between medicolegal identifiers and study codes will be stored separately from the analytic dataset, with access restricted to authorized personnel. Analysis files will contain only study codes and variables required by this protocol. DICOM and image metadata will be reviewed for identifiers before transfer. Versioned data dictionaries, rating forms, code, and a protocol deviation log will be retained on institutionally approved storage. Public release of case-level forensic data or images will occur only if permitted by ethics, legal, and custodial requirements.

Contextual Variables

When lawfully available, the case form will capture age category, sex, tooth type and position, date and environment of recovery, estimated postmortem interval, decomposition and storage conditions, date of each examination, and cause and manner of death as documented in the official medicolegal conclusion. The official conclusion must have been established independently of the study scores. Missing contextual variables will be reported as missing and will not be reconstructed from names or locations.

Multimodal Workflow

After eligibility assessment and coding, the workflow will proceed from the least destructive to the most destructive modality: photographic record review, CBCT remeasurement, and histological section review. Measurement files will use randomized specimen codes. Raters for each modality will remain unaware of measurements from the other modalities and of cause-of-death information until all modality-specific ratings have been locked.

Standardized Photography

Existing photographs were acquired using a Canon EOS 60D camera (Canon Inc) under controlled illumination, against a neutral background, and with fixed specimen geometry. The American Board of Forensic Odontology number 2 scale provides a size and geometric reference; it is not a colorimetric standard. Available native image and Exchangeable Image File Format (EXIF) metadata will be retained. Because a validated color target and a fully color-managed workflow were not used consistently, Delta E and other device-dependent color difference metrics are outside the scope of this protocol [21-24].

Two calibrated dental raters will independently score each randomized image using the Score of Pink Teeth Color (SPTC) scale (1="faint pink," 2="pink," and 3="dark pink") and the Score of Pink Teeth Region (SPTR) scale (1="root just below the cervical region," 2="cervical half of the crown," and 3="incisal half of the crown") [13,14]. Images with uncontrolled glare, clipped exposure, an absent scale, or an orientation that prevents region assignment will be classified as nonevaluable for the affected score. Calibration will use reference images that are not included in the analytic set.

CBCT Structural Assessment

Original DICOM data will be used whenever available. Scanner model, field of view, voxel size, tube voltage, tube current, exposure time, and reconstruction software will be exported from DICOM headers or contemporaneous acquisition records. The tooth will be reoriented along its long axis. At a plane 1 mm apical to the cementoenamel junction, raters will identify the outer tooth boundary and pulp-space boundary. Radial dentin thickness will be measured in the buccal or labial, lingual or palatal, mesial, and distal directions. The primary CBCT outcome is mean cervical radial dentin thickness in millimeters; secondary outcomes are minimum thickness and the pulp-to-tooth cross-sectional area ratio at the same plane.

Two oral and maxillofacial radiology raters will independently measure every evaluable tooth using the same software version and display protocol. Each CBCT rater will independently remeasure the same 5 randomly selected teeth after an interval of at least 14 days to assess intrarater reliability. CBCT gray values vary across devices and acquisition settings and will not be converted to Hounsfield units or interpreted as hemoglobin concentration, tissue density, or erythrocyte spread without an external calibration method [25].

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Histological Assessment

The supplied laboratory working procedure has been formalized as the approved SOP-PT-HISTO-001, Revision 1.0, which governs protocol-specified histopathological processing, when required, hematoxylin-eosin staining, documentation, quality control, specimen-level traceability, and blinded histological assessment. The predecessor procedure specifies fixation in 10% neutral buffered formalin and decalcification until the tooth is soft in a solution containing 0.3 M aluminum chloride, 3% hydrochloric acid, and 5% formic acid. It then uses 70% ethanol as a stopping point, followed by running tap water; dehydration in 80% and 95% ethanol and 2 changes of absolute ethanol for 1 hour each; clearing in 2 changes of xylene for 30 minutes each; 3 paraffin impregnations for 1 hour each; paraffin embedding; and microtome sectioning at 3 µm.

For hematoxylin-eosin staining, the predecessor procedure specifies deparaffinization in 3 changes of xylene for 30 minutes each; rehydration through absolute, 95%, 80%, and 70% ethanol for 3 minutes at each step; running tap water for 5 minutes; distilled water for 5 minutes; Mayer hematoxylin (Lillie's modification; ScyTek Laboratories Inc) for 5 minutes; running tap water for 2 minutes; distilled water for 5 minutes with microscopic inspection; water-soluble eosin Y (E-511; Fisher Scientific) for 1 minute; and distilled water for 5 minutes. Poststain dehydration uses 1 dip in each of 2 changes of 95% ethanol and 3 changes of absolute ethanol, followed by 3 xylene-clearing steps of 10 minutes each and Entellan mounting medium.

The predecessor procedure does not specify fixation duration, a timed or chemically verified decalcification end point, paraffin temperature, block orientation, slide preparation conditions, or microscopy settings. These undocumented historical parameters will not be reconstructed. For any prospective protocol-locked histopathological processing, applicable laboratory operating parameters—including fixation acceptance criteria, the decalcification end point method, paraffin processing temperature or temperature range, slide preparation conditions, and microscopy or scanning settings—will be documented and authorized before the relevant procedure is initiated. For existing slides and blocks, compliance with the current standard operating procedure (SOP) will not be assumed; specimen-level records will document only information supported by contemporaneous records or directly verifiable source materials, including fixation, decalcification, embedding or orientation, section thickness, staining batch, microscopy or scanning information, and magnification, in accordance with Biospecimen Reporting for Improved Study Quality principles [26]. Histology will be classified as nonevaluable when missing processing information or technical artifacts prevent reproducible assessment of the pulp-dentin interface. The approved SOP and a blank specimen processing or evidence record will be submitted as supplementary methods material (Multimedia Appendix 1).

For each eligible and technically evaluable histological specimen, the slide or section used for protocol-locked assessment will be selected before scientific scoring based solely on technical quality and anatomical completeness, without reference to the presence, absence, or extent of the histological finding being scored. Both raters will evaluate the same selected slide or section. The root, cervical, and crown regions will each be divided radially into zone 1 (peripulpal dentin), zone 2 (middle dentin), and zone 3 (peripheral dentin). Two blinded oral biology or pathology raters will independently record the farthest dentinal zone containing recognizable erythrocytes or eosinophilic or pigmented material morphologically compatible with blood-derived products: 0="none," 1="zone 1 only," 2="up to zone 2," and 3="up to zone 3." Staining precipitate, tissue folds, sectioning artifacts, chatter, tears, mounting artifacts, and decalcification artifacts will not be counted as positive findings. Disagreements will be retained for reliability analysis before a consensus value is produced for descriptive cross-modal analysis. Routine hematoxylin-eosin morphology will not be described as chemical confirmation of hemoglobin [7,27].

Protocol-Specified Outcomes

Table 2 summarizes the protocol-specified outcomes, their corresponding measurement scales, and their roles in the planned analyses.

Table 2. Protocol-specified outcomes and analysis roles.

Domains	Outcome	Scale and role
Feasibility	Technical evaluability by modality	Binary (yes or no); primary feasibility outcome
Photography	Pink color and region	Score of Pink Teeth Color (1-3) and Score of Pink Teeth Region (1-3); ordinal
CBCT[a]	Mean cervical radial dentin thickness	Millimeters; primary CBCT outcome
CBCT	Minimum cervical radial dentin thickness and pulp-to-tooth area ratio	Continuous; secondary CBCT outcomes
Histology	Farthest positive dentinal zone	Ordinal (0-3) for each of the root, cervical, and crown regions
Reliability	Agreement between raters	Weighted kappa or intraclass correlation coefficient with 95% CI
Integration	Association between modalities	Exploratory Spearman rank correlations with bootstrap 95% CI

[a]CBCT: cone-beam computed tomography.

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Rater Training, Blinding, and Data Quality

Before rating, each modality team will complete a written manual and a calibration exercise using nonanalytic examples. Specimen order will be randomized independently for each rater. Data entry ranges and units will be checked automatically, and a second person will compare a random 20% of entries with the source record. Any correction will retain the original value, reason, date, and person making the change. The analysis dataset will be frozen only after eligibility, linkage, and quality flags have been resolved.

Sample Size

Postmortem pink teeth are rare, and the study uses all authorized and technically eligible candidate specimens within the approved scope. No formal power calculation will be used to imply confirmatory inference. Precision will be communicated through 95% CIs and explicit denominators. The numbers of unique decedents, teeth per decedent, and evaluable teeth per modality will be reported. The small sample size is expected to limit the precision of reliability and cross-modal association estimates and may result in wide CIs. Accordingly, these analyses will be interpreted as descriptive or exploratory rather than confirmatory.

Statistical Analysis

The tooth is the measurement unit. The decedent is the inferential clustering unit when more than 1 tooth originates from the same person. Categorical variables will be summarized using counts, percentages, and denominators; continuous variables will be summarized using medians, IQRs, and ranges, with means and SDs provided when informative. Feasibility will be expressed as the proportion of eligible candidates yielding an evaluable result for each modality, with exact or Wilson 95% CIs.

Interrater agreement for the SPTC, SPTR, and histological zone scores will be assessed using linearly weighted kappa coefficients with 95% CIs. Agreement for continuous CBCT measurements will be quantified using a 2-way random-effects, absolute-agreement, single-measure intraclass correlation coefficient (ICC(2,1)). Cross-modal associations will be assessed using Spearman rank correlations, with 95% CIs obtained through a cluster bootstrap that resamples decedents. Candidates without verifiable anonymous decedent linkage will not enter the final analytic cohort; therefore, cluster-aware cross-modal analyses will be performed only among eligible candidates with confirmed decedent linkage.

No missing values will be imputed. Every analysis will report its available-case denominator and reasons for nonevaluability. No observation will be removed solely because it is statistically extreme. Cross-modal analyses are exploratory; exact *P* values, when shown, will be interpreted alongside effect sizes and CIs rather than against a binary significance threshold. No diagnostic classification or cause-of-death model will be fitted. Analyses will be performed using versioned R (R Foundation for Statistical Computing) or Python (Python Software Foundation) code retained with the study record.

Results

Study Status and Timeline

As of October 2, 2026, the working catalog contains 25 candidate teeth collected between 2023 and 2025. Research ethics approval by Ladokgi RE Martadinata (004123/KEP Ladokgi R.E. Martadinata/2026) is valid from July 29, 2026, to July 29, 2027, and describes a study scope involving 25 pink-teeth samples. Existing photographic and CBCT records are treated as historical source materials and are not automatically considered protocol-locked outcomes. Histopathological source materials will be evaluated according to their documented historical status and available processing records. Acquisition records are available for candidate specimens, but documentary eligibility adjudication, protocol-locked remeasurement, blinded histological outcome assessment, and integrated analysis have not yet been completed.

Outcome-blind documentary eligibility assessment of all 25 candidate teeth, including verification of applicable ethics coverage, provenance, medicolegal custody, and anonymous decedent linkage, is scheduled to take place from October 2, 2026, to October 16, 2026. Modality-specific technical evaluability assessment and resolution of outstanding documentary or technical queries are scheduled for October 19, 2026, to October 30, 2026. Final inclusion or exclusion status, the final analytic sample size, the number of unique decedents, and modality-specific evaluability status are scheduled to be locked on November 2, 2026.

Protocol-locked photographic and CBCT remeasurement, histology processing record verification, and blinded histological scoring are scheduled to take place from November 3, 2026, to December 4, 2026. Final resolution of measurement and quality control queries is scheduled for December 7, 2026, to December 18, 2026. The final analysis dataset is scheduled to be frozen on December 21, 2026. Reliability assessment and integrated cross-modal statistical analysis are scheduled to take place from December 22, 2026, to January 8, 2027. Preparation of the subsequent results manuscript is scheduled for January 11, 2027, to January 29, 2027. No protocol-locked scientific outcomes are reported in this protocol paper.

The study status and planned milestones are summarized in Table 3.

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Table 3. Study status and planned timeline.

Milestones	Status at current protocol revision	Planned timing
Candidate catalog and source record inventory	Available for all 25 candidate teeth	Completed
Documentary eligibility assessment, including verification of ethics coverage, provenance, medicolegal custody, and anonymous decedent linkage	Pending for all 25 candidate teeth	October 2, 2026, to October 16, 2026
Modality-specific technical evaluability assessment and resolution of documentary or technical queries	Pending	October 19, 2026, to October 30, 2026
Final cohort adjudication and confirmation of the final analytic sample size and number of unique decedents	Pending	November 2, 2026
Protocol-locked photographic and cone-beam computed tomography remeasurement	Pending	November 3, 2026, to December 4, 2026
Histology processing record verification and blinded scoring	SOP-PT-HISTO-001, Revision 1.0 approved; specimen-level verification and scoring pending	November 3, 2026, to December 4, 2026
Final quality control review and resolution of outstanding measurement queries	Not started	December 7, 2026, to December 18, 2026
Final dataset freeze	Not started	December 21, 2026
Reliability assessment and integrated statistical analysis	Not started	December 22, 2026, to January 8, 2027

Reporting

The results article will follow the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement, including the relevant Biospecimen Reporting for Improved Study Quality elements for specimen handling [26,28]. A specimen flow diagram will show the numbers of candidate teeth and unique decedents, exclusions, and modality-specific denominators. All deviations from this version of the protocol will be dated and justified.

Discussion

Anticipated Findings

The study is expected to provide descriptive estimates of modality-specific technical evaluability and measurement reliability, together with exploratory cross-modal associations. Given the small convenience sample, potential clustering of multiple teeth within decedents, variable preanalytic conditions, and the absence of a representative pink-negative comparator, these estimates may be imprecise and will not support diagnostic or causal conclusions.

Anticipated Contribution

This protocol converts a visually striking but nonspecific observation into a traceable measurement workflow. Its main contribution is methodological: consistent photographic categories, anatomically defined CBCT measurements, conservative histological scoring, independent rating, and explicit denominators. The workflow may support more comparable case documentation and reduce the risk of overstating the significance of postmortem pink teeth in forensic opinions.

Strengths and Limitations

Strengths include multimodal assessment of the same teeth, independent blinded ratings, a locked data dictionary and amendment log, and separation of structural, morphological, and color evidence. The main limitations are convenience sampling, a small sample with potentially clustered observations, variable preanalytic conditions, incomplete historical acquisition metadata, and the absence of a representative pink-negative comparator. Selection based on the presence of visible pink discoloration prevents estimates of prevalence or diagnostic accuracy. Although the renewed ethics approval describes the study as involving 25 pink-teeth samples, the final analytic cohort may still be reduced by unresolved issues involving provenance, medicolegal custody, decedent linkage, processing documentation, or modality-specific technical limitations.

Comparison With Prior Work

Previous investigations of postmortem pink teeth have used heterogeneous approaches to visual description, imaging, and histological assessment. Recent reviews continue to emphasize the nonspecific nature of the finding and the limitations of interpreting it as a stand-alone indicator of drowning or another cause of death [10-12,17]. The present protocol differs by prioritizing standardized multimodal measurement, independent rating, explicit eligibility criteria, and measurement reliability rather than diagnostic classification.

Future Directions

Future studies should evaluate the reproducibility of this workflow in larger, independent, and preferably multicenter datasets with explicit decedent-level linkage and standardized acquisition procedures. External validation and appropriately selected comparison groups would be required before any diagnostic or population-level interpretation could be considered.

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Dissemination Plan

Following completion of the eligibility assessment, protocol-locked assessments, reliability analysis, and final dataset freeze, the findings will be disseminated in a subsequent results manuscript. Nonidentifying protocol materials, the data dictionary, and analysis code may be shared, subject to applicable ethics, legal, and custodial restrictions.

Conclusions

A conservative data-existing protocol may enable multimodal characterization of postmortem pink teeth, provided that specimen authorization, decedent linkage, and measurement reproducibility are established before integrated analysis. The study is designed to standardize evidence documentation and generate hypotheses, not to diagnose drowning or another cause of death.

Acknowledgments

The authors acknowledge the forensic, radiology, histology, and institutional personnel who maintain the specimen custody and technical records required for this study.

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Data Availability

The protocol, data dictionary, and nonidentifying analysis code will be shared with the results report. Individual-level forensic records, Digital Imaging and Communications in Medicine (DICOM) data, histology images, and photographs are not publicly available because they may contain identifiable information or be subject to medicolegal custody restrictions. Qualified access may be considered only with the necessary ethics, legal, and custodian approvals.

Generative AI Use

The authors declare that generative AI was used solely for language editing to improve grammar, clarity, and readability. It was not used to generate or analyze study data, make methodological decisions, interpret findings, or formulate scientific conclusions. All AI-assisted edits were critically reviewed and approved by the authors, who take full responsibility for the final manuscript. The language-editing tools used were ChatGPT (OpenAI) and Grammarly (Grammarly, Inc.).

Authors' Contributions

Conceptualization: AFH, ER
Data curation: AFH
Investigation: AFH
Methodology: AFH, ER, FO, ES
Project administration: AFH
Supervision: ER, FO, ES
Validation: ER, FO, ES
Visualization: AFH
Writing—original draft: AFH
Writing—review and editing: AFH, ER, FO, ES
All authors remain accountable for the work.

Conflicts of Interest

None declared.

Multimedia Appendix 1

Approved histopathological standard operating procedure (SOP-PT-HISTO-001, Revision 1.0), including blank specimen processing, quality-control, and eligibility documentation forms.
[PDF File (Adobe PDF File), 469 KB-Multimedia Appendix 1]

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Abbreviations

CBCT: cone-beam computed tomography
DICOM: Digital Imaging and Communications in Medicine
EXIF: Exchangeable Image File Format
ICC(2,1): 2-way random-effects, absolute-agreement, single-measure intraclass correlation coefficient
SOP: standard operating procedure
SPTC: Score of Pink Teeth Color
SPTR: Score of Pink Teeth Region
STROBE: Strengthening the Reporting of Observational Studies in Epidemiology

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