

Diagnostic Accuracy of Cold, Heat, and Electrical Pulp Tests: An In Vivo Study

Short Title: Diagnostic Accuracy of Pulp Sensibility Tests

Institution / Sponsor	Istanbul University Faculty of Dentistry
Study Site	Department of Endodontics, Istanbul University Faculty of Dentistry Istanbul, Türkiye
Ethics Approval	Ethical Committee Ref. 2024/48 Rev-2 (05.06.2024)
TİTCK Approval	Protocol No. 2023/38
ClinicalTrials.gov ID	NCT Number: [Not yet assigned]

Document Date: 05.06.2024

Study Protocol

Background and Rationale

Accurate assessment of pulp tissue status is essential for endodontic diagnosis and appropriate treatment planning. Pulp sensibility tests -including cold, heat, and electric pulp testing- are widely used in routine clinical practice; however, their performance may vary across clinical conditions and patient populations. Direct assessment of pulpal bleeding during access cavity preparation is commonly considered a practical reference for pulp vitality in clinical settings (1-7). This study evaluates the diagnostic performance of commonly used pulp sensibility tests against pulpal bleeding as the reference standard in an endodontic clinical population.

Study Objectives

Primary Objective

To evaluate the predictive performance of cold, heat, and electric pulp sensibility tests for determining pulp vitality, using pulpal bleeding during access cavity preparation as the reference standard.

Secondary Objectives

- To quantify false-positive and false-negative responses for each test relative to the reference standard.
- To estimate sensitivity, specificity, and overall accuracy for each test.
- To assess agreement between each index test and the reference standard using Cohen's kappa.
- To compare test results across cold, heat, and EPT using appropriate paired statistical methods.

Study Design

Prospective, observational, in vivo diagnostic accuracy study conducted in routine care at a university endodontics clinic. Participants are not assigned to interventions; index tests are applied as standardized diagnostic procedures.

Study Population and Setting

Participants are selected from patients presenting to the Endodontics Clinic at Istanbul University Faculty of Dentistry, including patients referred from the Department of Oral and Maxillofacial Radiology, who are evaluated in routine care and determined to require endodontic treatment for at least one tooth.

Eligibility Criteria

Inclusion Criteria

- Age ≥ 18 years
- At least one tooth indicated for root canal treatment (primary treatment or nonsurgical retreatment), confirmed at the initial examination unit
- Able and willing to provide written informed consent
- One tooth per participant included

Exclusion Criteria

Tooth-related: full-coverage crowns, extensive restorations affecting testing area, history of trauma (study tooth), root resorption, incomplete root development, fractures/cracks, regressed pulp chambers or calcified root canals.
Patient-related: severe or uncontrolled systemic disease (ASA physical status 3–6).

Sample Size

Sample size was determined using the mean prevalence reported in comparable diagnostic accuracy studies of pulp sensibility tests (assumed prevalence: 45%) (2, 4, 5, 8-11) . With 95% confidence, expected sensitivity/specificity of 95%, and a 5% margin of error, the minimum required sample was 163 teeth. To allow for potential exclusions and to improve precision, a total of 175 participant were included in the study, with one tooth per participant selected.

Recruitment and Consent

Eligible participants were identified during routine clinical evaluation. According to the ethical principles of the Declaration of Helsinki, informed and written consent were obtained from the patients before the clinical tests.

Blinding and Roles

Index test operator: performs pulp sensibility tests; blinded to clinical signs/symptoms, dental history, and radiographic findings; and blinded to any prior sensibility test results obtained during routine evaluation.

Reference standard assessor: prepares the acces cavity and assesses pulpal bleeding; blinded to index test results.

Assessors are blinded to each other's findings.

Test Standardization

- Teeth were isolated with cotton rolls and dried with an air syringe.
- Participants were intructed to raise their hand immediately upon perceiving pain, discomfort or tingling.

- Baseline response was obtained from an asymptomatic, functional contralateral tooth without prior endodontic treatment (or an adjacent tooth if contralateral is unavailable). The test tooth was tested last.
- Pulp tests were applied to standardized areas: incisors/canines (middle third buccal), premolars (occlusal third), molars (mesiobuccal cusp tip area or closest available site).

Pulp Sensibility Tests

Order of tests: Cold → Heat → Electric pulp test, with a 5 minute interval between tests.

Cold Pulp Testing (Refrigerant Spray)

The cold test was performed by using a refrigerant spray containing propane-butan mixture (Endo-Frost; Roeko, Coltene Whaledent, Germany). Cold spray was applied to a size 2 cotton pellet, and once visible frosting was observed, it was placed on the tooth surface. The cotton pellet was held in contact for up to 15 seconds or until the patient responded.

Heat Pulp Testing (Frictional Heat)

The heat test was performed using a sterile rubber cup (NAIS, Sofia, Bulgaria) mounted on a low-speed contra-angle handpiece at a constant speed setting. The rubber cup was applied with light, consistent contact to predefined areas of the tooth surface without water cooling, and the stimulus was stopped when the patient responded or after a maximum of 7 seconds.

Electric Pulp Testing

The electric pulp test was performed using an apex locator integrated with a built-in pulp tester (Ai-Pex; Woodpecker, Guilin, P.R. China). A small amount of toothpaste was applied to the 2 mm electrode tip before placing it on the predefined area on the tooth surface. The device was set to the lowest current increase rate to precisely determine the numeric value at which the patient first perceived the stimulus. This value was recorded. The contralateral tooth was used as a control. If the response from the test tooth was equal to or less than the control tooth, the test result was recorded as 'response present.' If the response was higher or no response was observed up to the maximum value of 80, the result was recorded as 'response absent.'

Reference Standard: Pulpal Bleeding During Access Cavity Preparation

Immediately after completion of the pulp tests, local anesthesia (Ultracain D-S Forte, Sanofi, Paris, France) was administered to the teeth, and the teeth were isolated with rubber dams. A standard endodontic access cavity was prepared by a different researcher who was blinded to the pulp test results. The presence or absence of pulpal bleeding in the pulp chamber was recorded by direct visual inspection under 3.5x magnification (dental loupes). Teeth were classified as vital when bleeding was observed within the pulp chamber. Teeth were classified as non-vital when no bleeding was present in the pulp chamber. In cases where bleeding was observed only within the root canal(s) without bleeding in the pulp chamber, or where

bleeding was not present in all canals of a multi-rooted tooth, the tooth was classified as partially necrotic and recorded as non-vital. Following determination of pulp status, root canal treatment was performed. Teeth with uncertain pulp status were excluded from the study. As the bleeding status of the pulp were recorded as present or absent, no indeterminate test result category was defined or analyzed in this study.

Data Collection and Management

- Pulp test outcomes were recorded dichotomously (present/absent). Electric pulp test threshold value was additionally recorded.
- Reference standard outcome was recorded as bleeding present/absent and final classification vital/non-vital.
- Data were stored in a secured dataset accessible only to the research team. Identifiers were minimized; any exported documents may be redacted for confidentiality.

Risks, Burden, and Safety

Pulp tests may cause transient pain/discomfort. Local anesthesia and access cavity preparation were performed as part of indicated clinical care. Any adverse events or unexpected issues were managed according to standard clinical protocols and documented as appropriate.

Statistical Analysis Plan (SAP)

The statistical analyses and decision rules described below were pre-specified before study initiation and the SAP was finalized prior to database lock and before any outcome analyses were performed. Data were analyzed in IBM SPSS Statistics (Version 23). One tooth per participant was included. Teeth with uncertain reference standard classification were excluded from analysis. Index test results (cold, heat, and electrical pulp test) were coded as binary outcomes (response present/absent). The reference standard was pulpal bleeding during access cavity preparation, coded as vital (bleeding present) or non-vital (bleeding absent). Partial necrosis (bleeding limited to root canals without pulp chamber bleeding and/or not present in all canals) was classified as non-vital.

For each index test, 2×2 contingency tables were constructed to identify true positives, false positives, true negatives, and false negatives. Primary performance measures were PPV and NPV with 95% confidence intervals (Wilson score method). Secondary measures included false-positive and false-negative proportions, sensitivity, specificity, and overall accuracy (all with 95% Wilson CIs), as well as prevalence of vitality based on the reference standard. Agreement between each index test and the reference standard was assessed using Cohen's kappa. Differences among the three tests were assessed using Cochran's Q test, and pairwise comparisons were conducted using Bonferroni-adjusted Z tests. Statistical significance was set at $p < 0.05$.

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Participant Information Sheet and Informed Consent Form

INFORMED CONSENT FORM

Version date: 05.06.2024 Version no: 02

The title of this study is “Diagnostic Accuracy of Cold, Heat, and Electric Pulp Tests: An In Vivo Study”. This is a specialty (residency) thesis research project. The aim of the study is to determine the predictive values and accuracy of thermal (heat/cold) and electric pulp tests (a device that tests tooth vitality by delivering specific signals to the tooth) in assessing the vitality of the dental pulp, which is the living tissue inside the tooth.

Root canal treatment is performed when there is deep decay in a tooth or when the tooth is infected. In this research method, tests used to determine the vitality status of teeth that have already been indicated for root canal treatment will be performed. These tests are thermal (cold and heat) and electrical tests. The tests specified in this study will be applied to your tooth/teeth, and afterwards root canal treatment will be performed as part of your routine care (outside the research procedures). All of the tests specified are tests that are successfully used in routine clinical practice.

In this study, pulp sensibility tests will be applied in sequence to the tooth for which root canal treatment has already been indicated. All pulp sensibility tests (cold, heat, and EPT) will be performed first on an asymptomatic, functional contralateral tooth without previous endodontic treatment (or, if unavailable, on a suitable adjacent tooth) to establish a baseline response before testing the test tooth. First, a cold spray test (application of a cold agent sprayed onto sterile cotton), then a frictional heat test (stimulation of the living tissue by generating heat on the tooth with a rotating rubber cup), and finally an electric pulp test will be applied. A 5-minute interval will be allowed between tests so that the results do not affect each other. Your tooth will first be isolated from oral fluids (with cotton rolls and an air spray). The cold spray will be sprayed onto a small piece of cotton and placed on the front middle surface of your tooth and applied for a maximum of 15 seconds. Then, a polishing rubber cup (used for polishing dental fillings) will be rotated on your tooth for a maximum of 7 seconds. Finally, after applying a thin layer of toothpaste to the tooth as a conducting medium, the electric pulp test probe will first be placed on the front middle surface of the tooth opposite the tooth to be tested, and electrical current will be started at the lowest level. Based on the numerical value displayed by the device and your perceived pain/discomfort, the numerical value will be recorded; then, according to this value, the electric pulp test will be applied to the tooth to be tested in the same way. In all three tests, the test will be stopped and the results will be recorded as soon as you feel any pain/discomfort/tingling. If you do not feel any pain/discomfort/tingling within the specified time, the test will still be stopped and your test result will be recorded as ‘response absent’. After the tests are completed, an access cavity (opening) used in routine treatment will be prepared and your test result will be compared

with the bleeding status of your tooth. After preparation of the access cavity and recording of vitality status, standard root canal treatment will be performed for your tooth/teeth.

Possible undesired effects and risks are as follows: The tests to be applied do not cause harm to the teeth or surrounding tissues.

At the end of the study, the responses of three different tooth sensibility tests that are routinely used in dentistry will be evaluated in different clinical conditions, and it will be compared which test yields results closer to the diagnosis. Thus, it will be determined which of these routinely used tests is more helpful for diagnosis. This study may provide guidance regarding the comparison and accuracy of tests that clinicians can use to establish a correct diagnosis in the clinic.

The study duration is 6 months. The expected participation time for volunteers is approximately 1 hour (time to complete the tests and the subsequent root canal treatment). The institution where the study will be conducted is the Department of Endodontics, Istanbul University Faculty of Dentistry. A total of 175 volunteers will be included in the study.

There are alternative treatments outside the research method; namely, root canal treatment can be performed on the tooth/teeth already indicated for root canal treatment without applying the above tests.

Your participation in the research is voluntary. You may refuse to participate or withdraw from the study at any time without any penalty or sanction and without losing any of your rights. If you do not accept participation or if you are removed from the study program for any reason, there will be no disruption in your treatment. Records that could reveal your identity will be kept confidential, will not be disclosed to the public, and the study results will not be published in a way that could lead to identification. If such a situation arises, your additional consent will be obtained. Even if the study results are published, your identity will remain confidential. Monitors, auditors, the ethics committee, the institution, and other relevant health authorities may have direct access to the volunteer's original medical records; however, this information will be kept confidential. If new information related to the research that may affect your willingness to continue participation is obtained, you or your legal representative will be informed. You will not incur any financial responsibility for study-related expenses, and no payment will be made to you.

Dear Dr. Ecem Erden, I have been informed that a medical research study will be conducted at the Department of Endodontics, Istanbul University Faculty of Dentistry, and the above information regarding this research has been provided to me. After receiving this information, I have been invited to participate in such a study as a "volunteer". If I participate in this study, I believe that great care and respect will be shown for the confidentiality of my personal information that should remain between my physician and me. I have been given sufficient assurance that my personal information will be protected with due care during the use of study results for educational and scientific purposes.

During the conduct of the project, I may withdraw from the study by informing the researchers without stating any reason. In addition, I may be excluded from the study by the researcher provided that no harm is caused to my medical condition.

I will not assume any financial responsibility for the study-related expenses. No payment will be made to me.

The necessary assurance has been provided that, should any health problem occur for any reason directly or indirectly arising from the research procedures, all required medical interventions will be provided (and I will not bear any financial burden related to these medical interventions).

If I experience any health problem during the study, I know that I can contact Dr. Ecem Erden at any day and time at the following address and phone number: Istanbul University Faculty of Dentistry, Süleymaniye, Prof. Dr. Cavit Orhan Tütengil St. No:4 D:6, 34116 Fatih/Istanbul, Türkiye. Phone: +90 5***.

I am not obliged to participate in this research and I may choose not to participate. I have not encountered any coercive behavior regarding my participation. If I refuse to participate, I know that this will not harm my medical care or my relationship with my physician.

After having sufficient time to consider, I have decided on my own to take part in the above-mentioned research project as a “volunteer”. I accept this invitation of my own free will, having been adequately informed about the outcomes and effects of the research, without any pressure, and without any promise of benefit.

A copy of this signed form will be given to me.

I have read all explanations in this informed consent form. Written and verbal explanations about the research described above were provided to me by the physician named below. I know that I am participating voluntarily, without any pressure or coercion, and that I may withdraw from the study at any time with or without providing a reason.

Volunteer’s Full Name / Signature / Date

Name / Signature / Date of a competent researcher in the study team

Name / Signature / Date / Address / Phone of the witness to the consent process (if required)

Legal representative (mother/father) – Full Name / Signature / Date / Address / Phone (if required)