F22 Age Estimation: New Methodology to Assess Aspartic Acid Racemization in Teeth

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After attending this presentation, attendees will have a better understanding of a simplified aspartic acid racemization age estimation method comparing the use of whole teeth or whole teeth with enamel and cementum removed. The amino acid enantiomer detection utilizes high performance liquid chromatography (HPLC) coupled with mass spectrometry (MS).

This presentation proposes a method to estimate ages that may benefit humanity and science by improving the accuracy and reducing the ranges of ages estimated. This improvement will impact the forensic science community by providing medical examiners and coroners better information to identify unknowns and allow for more discrete search parameters for matches between missing person and unidentified body databases.

In nature, amino acids are primarily synthesized as levorotary or L- isomers. Spontaneous conversion over time by a process known as racemization converts some of the L-form of amino acids to the D-form resulting in a mixture of the L- and D-forms. These stereoisomers are detectable mirror image enantiomers. It is well established that an age- dependent racemization occurs in various human and animal tissues, including the white matter of the brain, the lens of the eye, the aorta, cartilage, skin, bone, and both tooth enamel and dentin. It is possible to calculate and use the ratio of the L and D forms, in long-lived proteins that are metabolically stable, to estimate the age of the individual at death or the age of a living individual.

The accuracy, reproducibility, relative simplicity of methodology, and time required make tooth enamel and dentin among the best target tissues for age estimation using these methods. Among the amino acids tested, aspartic acid appears to give the most reliable results. This method offers the potential for the most accurate age estimations with the smallest ranges for all age groups. (+/- 3-4 years).

Gas chromatography (GC) and high performance liquid chromatography (HPLC) coupled with detection of fluorescence methods have been reported. Different teeth and different portions of teeth have been tested. Since dentin and enamel form at different times for different teeth, aspartic acid racemization will vary for different teeth and vary at different locations of a single tooth. Most researchers currently recommend sectioning teeth from labial to lingual and using a full length section from near the center of the tooth.

The current study will investigate different methodology in both preparation of the teeth and detection of the enantiomers. Researchers report that the complexity of preparing the teeth seems to be the most likely cause of variations in their results. When possible, teeth were tested in homonymous pairs from the same individual of known age at death. The procedure in this study was designed to minimize the handling and manipulation of the teeth. Excessive processing may generate heat and accelerate racemization. Minimal sectioning and grinding was accomplished using water-cooled instruments. Single rooted teeth were tested in two forms, either intact with no mechanical processing or with the enamel and cementum layers removed using water-cooled, rotary dental instruments. If no homonymous multi-rooted teeth were available, the target teeth were sectioned longitudinally into mesial and distal "halves", and one of the halves stripped of enamel and cementum.

Samples were frozen and pulverized in a Model 6750 CertPrep freezer mill and the resultant powder stored in appropriately labeled sterile containers. The tooth powder samples were demineralized with pH adjusted Na_2EDTA in 2 mL centrifuge tubes. The washed sediment was transferred to another tube and hydrolyzed for six hours with 6 M hydrochloric acid. After drying, the residue was derivatized using Marfey's reagent to produce chromatographically distinct L and D forms of aspartic acid. The analysis was performed by LC/MS/MS in positive ion mode using an Applied Biosystem 3200 Q-Trap mass spectrometer with an Agilent 1100 HPLC. The column used was a Phenomenex Synergi Polar RP, 50x2 mm, 4 micron. Three MRM transitions were monitored for each of the enantiomers of aspartic acid, 386 to 341.2, 386 to 144.2 and 386 to 185.8 amu.

This study examined new methods both in preparation and analysis. The study compares results from tests performed on processed and unprocessed teeth. If accurate results can be obtained from unprocessed or minimally processed teeth using standardized analysis procedures, many of the problems associated with widely varying techniques for assessing aspartic acid racemization for age estimation can be minimized. Consequently, the most accurate method with the smallest range for estimating ages for persons both living and deceased and of all ages can become more available to investigators.

Age Estimation, Aspartic Acid Racemization, HPLC/Mass Spectometry

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