H90 Test of Osteon Circularity as a Method of Human/Non-Human Identification

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After attending this presentation, attendees can expect to learn how new osteon area and circularity tests can help with the identification of fragmentary remains and how to utilize the proposed method on fragmentary skeletal casework.

This presentation will impact the forensic science community in terms of knowledge, competence, and performance by describing to the attendees how osteon measurements can be used to distinguish between human and non-human fragmentary remains, educating the attendees on the proper method of application of the proposed method, and providing a standard operating procedure for the implementation of this method in forensic practice.

Due to increased need for the identification of highly fragmentary skeletal remains, more laboratories are turning to histological techniques for fast, accurate species identification tests. This research adds to the body of knowledge by focusing on the overall shape and areas of the osteons. Human bone specimens from known individuals were utilized to develop reference data. Each of the limb bones from five individuals was histologically sampled, yielding slides from 30 specimens. Digital images of a random location on each slide were captured at 100x magnification using a color digital camera.

Osteon area was measured with Image J program (NIH). The program requires the perimeter of each osteon be traced. The perimeter outline provides the basis for calculation of area and other measurements. The assessment of shape, called the circularity index (CI), was obtained using Image J's circularity measurement option. Area and circularity data were obtained for 1204 osteons from the 30 samples from five individuals to form the reference data set. These individuals were selected from the Bass Collection (UTK). The same procedures were used to obtain area and circularity from 1,442 osteons in 37 samples from 35 individuals from JPAC-CIL (calculating the mean values for area and circularity from a random sample of 30 osteons drawn from each sample image). This mean is compared to the mean and "standard error of the mean" calculated from the reference data. A bootstrap step was taken to generalize the reference data and make the statistical tests meaningful (i.e., to avoid the trap with large sample sizes whereby any difference is statistically significant). The 1,204 osteon measurements for each variable had independent samples of size 30, randomly drawn 1,000 times. Each time a sample was selected, the mean was calculated. Specimen mean values were compared to the grand mean (e.g. mean of means) utilizing the standard deviation (SD) of the 1,000 means (bootstrap standard error of the mean) as the basis for a formal test of the null hypothesis that the case specimen is human (e.g. has osteons that are within the bounds of natural variation for area or circularity).

The grand mean value obtained for human osteons was 37,365 microns and the standard deviation of the bootstrap means was 2,728 microns. The distribution closely approximates normality. Consequently, it was decided to conduct a one-sided test of the null hypothesis (e.g., specimen is human) in the direction of smaller mean areas. The resulting cutoff values for osteon area were set as follows: mean < 31,909 for $p<0.025$; mean<29181 for $p<0.005$. The circularity values for humans deviate downward from perfectly circular (e.g., value of 1). The grand mean value was 0.94 and the SD of the bootstrap means was 0.0053. The distribution closely approximates normality. A two- sided test was used with the following referents: mean >0.96 or mean <0.92 for $p<0.01$; mean >0.95 or mean <0.93 for $p<0.05$.

Application of the two tests to the independent test sample (N=37) yielded no rejections of the null hypothesis for osteon area. For circularity, there were 17 (46%) rejections of the null at the $p<0.05$ (4 below and 13 above the mean) level and 4 (11%) rejections at the $p<0.01$ level (3 below and 1 above the mean). Of the 13 specimens above the mean in the $p<0.05$ level test, all but one were approximately the same value as the referent (e.g. 0.95). While the osteon area test meets or exceeds expectations, the circularity test did not perform as well. Two potential explanations, neither mutually exclusive, are plausible. First, the protocol for avoiding measurement of osteons that have been sliced at an oblique angle was not consistently followed between derivation of the reference data and the test data. This scenario is unlikely to be a sufficient explanation, since the same individuals measured both specimen sets and were trained by the senior author. Second, the reference data incorporated measurements from 5 individuals while the test sample included measurements from 35 different individuals. Thus, the test sample demonstrates variation among individuals that must be captured in the reference data for the test to be effective. The optimal solution is to incorporate the test data into the reference data prior to publishing the final recommended test.

Physical Anthropology, Bone Histology, Osteon Area and Circularity