



D6 Time Since Death Estimation From Gut Flora

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After attending this presentation, attendees will understand: (1) the principles of current time since death (TSD) investigation including the strengths and limitations; (2) how specific species of gut flora change over time; (3) the relationship between postmortem gut flora change and TSD; and, (4) how to practically utilize the method of estimation.

This presentation will impact the forensic science community by facilitating criminal investigation with a universal method of estimating TSD allowing for the removal of the body from original decomposition context as well as a sampling window encompassing various stages of decomposition

The method limits to stages of decomposition where intestines are present and not mummified. Because the gut flora of an individual impact the earlier stages of decomposition, monitoring the gut flora change over time can yield a formula to estimate TSD. This preliminary study uses 10 human individuals for the basis of the relationship between the change in postmortem gut flora and TSD. None of the individuals were buried, clothed, or covered in any artificial wrap. All individuals were placed in the supine position on the ground at the Anthropological Research Facility in Knoxville, TN, which consists of sparse forest. Individuals are sampled every three days in the first two weeks of May 2011, and due to temperature increase, are sampled once a day from the last two weeks of May 2011 to present. At each time point for each individual, three different samples are taken from the same location in the cecum and stored at -20° C until processing. After the gut flora from the cecum of the large intestine of deceased individuals is sampled, the DNA is isolated using the PowerSoil® DNA Isolation Kit, and specific, common species are amplified in real time with the use of quantitative, real time polymerase chain reaction (PCR) using primers for conserved, ribosomal genes (16S). The real time PCR bases the amplification of the selected bacterial species off a standard curve, which is developed by isolating an organism of the specific species and transforming it into a plasmid so the species chain can be sequenced or decoded, to confirm the correct organism is chosen. The confirmed organism is then run through real time PCR by serial dilutions, which creates the standard curve. The environmental samples compare to the standard curve to confirm the amplification of the correct organism based on size as well as compares to the standard curve to delineate the number of copies. The change in these species over time can be formulated with the known TSD to produce a standard relationship reproducible for popular use. The study also includes samples taken at known time points from previously un-sampled individuals to cross-verify the determined relationship between the specific species of gut flora and the TSD. A total of six human individuals are sampled for this blind study. The results of this study show observed gut flora change over time as decomposition conditions change resulting in an increase of some species and the decline of others. Shortly after death, the number of observed microbes increases exponentially and decreases in the later stages of decomposition as the intestines no longer remain and other, soil microbes continue with decomposition, or the body and organs mummify to provide an inhospitable environment for the specific flora. The study includes two organisms specifically to observe the phenomenon of microbial competition in the process of decomposition. After publishing, the method of TSD estimation also permits acceptance as expert witness testimony in legal contexts, because the method is based from empirical tests, the error rates are known, and the method uses previously published sampling and processing practices so is generally accepted within the relevant, scientific community. The purpose of the study is to formulate a legally legitimate method for the estimation of TSD so individuals of varying technical backgrounds can understand and easily interpret results. The results obtained from this study of 16 human individuals additionally serve to provide a foundation for future work relating microbial patterns to the practice of Forensic Anthropology; this provides empirical methods off which the field can build.

Time Since Death, Gut Flora, PCR