



### E31 Discrimination of Human and Animal Blood Traces Via Raman Spectroscopy

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After attending this presentation, attendees will become knowledgeable about how Raman spectroscopy coupled with chemometrics can be used to analyze, accurately identify, and discriminate between bloodstains from humans and 11 species of animals.

This presentation will impact the forensic science community by introducing a unique method for non-destructive bloodstain analysis and confirmatory identification, with a statistical level of confidence, which could potentially be performed at the crime scene.

The identification of a body fluid stain is an important and necessary aspect of many forensic investigations. Various presumptive tests are currently used for identifying a stain as blood; however, these tests are most commonly oxidation-reduction assays, which use hazardous chemicals and are destructive. Common confirmatory blood tests are microcrystal assays (e.g. Teichmann or Takayama assays). The Ouchterlony test, or similar immunochromatographic assays, can be employed to determine if the blood is non-human. It is ultimately preferable to confirm the presence of blood and the species of origin before forensic DNA profiling, but this can be practically problematic.

If a DNA profile is not extracted from the sample, then the suspected blood is usually presumed to be non-human and further characterization omitted. This is an erroneous testing scheme primarily because there is a lack of confidence that the sample is in fact blood, and of human origin, due to potential false positives. This approach could also be detrimental for crime labs since time and money would be wasted on non-human or non-blood samples. Since the amount of suspected blood evidence may be miniscule, it needs to be preserved and analyzed efficiently. Therefore, a non-destructive method to competently identify human blood would be highly valuable. Raman spectroscopy is a technique that has the potential as both a non-destructive confirmatory identification for blood and as a species of origin assay.

Raman spectroscopy has been proven as an effective and versatile analytical technique for a variety of forensic applications, including identification of lipsticks, drugs, explosives, paints, and fibers. Raman analysis often requires no sample preparation, is typically non-destructive, and has the ability to analyze microscopic amounts of sample. This technique is based on the detection of light that is inelastically scattered by a sample upon irradiation from a monochromatic light source. A Raman spectrum contains numerous distinctive bands which correspond to specific molecular vibrational modes. For blood in particular, Raman spectra provide rich detail and has been targeted in previous forensic studies. Popularity of Raman spectroscopy has been growing in forensic science especially due to reduced cost of instrumentation, including portable instruments, and its numerous possible applications.

This study has analyzed blood from twelve different species, including human. For forensic relevance, the animal (non-human) samples were selected to represent three groups: (1) animals that are bred for domestication (cat, dog, horse); (2) those consumed as food (chicken, cow, pig, rabbit); and, (3) those integrated with human existence (mouse, rat, opossum, raccoon). Analyzing a variety of animals' blood enhances the forensic practicality of the study and adds more certainty to model predictions. To account for the complexity of the dataset, Partial Least Squares Discriminant Analysis (PLSDA) classification models were utilized. The first PLSDA model differentiated between human and animal blood spectra in a binary fashion (human vs. animal).<sup>1</sup> The second PLSDA model was used to differentiate between human blood spectra and those from each species of animal surveyed.<sup>2</sup> To validate both models, classification predictions were made for a set of internal and external unknown samples. The constructed models exhibited a great ability to discriminate human from animal blood. This study demonstrated a comprehensive and robust method for analyzing a suspected bloodstain to identify human origin. The goal of the analytical approach is to be used for the rapid and non-destructive identification and characterization of a bloodstain at a crime scene.

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### References:

1. McLaughlin, G., Doty, K.C. & Lednev, I.K. Discrimination of human and animal blood traces via Raman spectroscopy. *Forensic Sci Int* 238, 91-95, (2014).
  2. McLaughlin, G., Doty, K.C. & Lednev, I.K. Raman Spectroscopy of Blood for Species Identification. *Analytical Chemistry* (2014), 86: 23, 11628-33.
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### Raman Spectroscopy, Blood, Human