

**Emily Miller**  
**Class of 2023**  
**Forensic Science Chemistry Concentration**  
**Effect of Hormone Replacement Therapy on Sex Determination Using Raman Spectroscopy**  
**Dr. Brooke Kammrath, Department of Forensic Science**

Raman spectroscopy is an emerging instrument within the field of forensic science due to its many advantages which include non-destructive and non-contact analysis both in the lab with benchtop instruments as well as the proliferation of field portable instruments indicating the potential for real-time crime scene analysis which would provide for potentially valuable investigative leads. There are a plethora of forensic applications of Raman spectroscopy currently employed, which includes the identification of illicit drugs, materials evidence (e.g., fibers, paint), and explosives. More recently, Raman spectroscopy has been investigated for the identification of different biological fluids (e.g., blood, semen, urine, saliva), in addition to making determinations of species, time since deposition, and biological sex [1]. Over the last 15 years, meaningful research has been published on the use of Raman spectroscopy for the determination or classification of sex from blood and dried blood deposits. It is known that there are differences in the makeup of blood between cisgender females and males such as variations in the coagulation factor V,  $\alpha$ 1-antitrypsin, and  $\beta$ 2- macroglobulin found in plasma [2]. Thus, the ability of Raman spectroscopy combined with chemometrics to differentiate blood samples by the biological sex of the donor is scientifically seen.

Hormone replacement therapy (HRT) is a common treatment for a variety of individuals, including women taking estrogens or progestogens to alleviate the symptoms of menopause, men taking testosterone to combat the natural decrease in its production with aging, and transgender or nonconforming individuals taking hormones to align their secondary sexual characteristics more closely with their gender identity. In the case of transgender hormone therapy, there are two types: masculinizing hormone therapy which provides androgens and antiestrogens to transgender men or transmasculine people and feminizing hormone therapy in which estrogens and antiandrogens are given to transgender women or transfeminine people. Given that HRT introduces exogenous hormones which will affect a person's biochemistry, especially in cases of transgender HRT, it is important to investigate the effects of HRT on the classification of sex by Raman spectroscopy. This research investigated how chemometric models that have been used for sex classification work when tested with samples from transgender individuals undergoing HRT.

Transgender and nonconforming individuals are disproportionately victims of violent crimes; however, they are not often included in scientific research, including forensic science research. It is essential to include this underrepresented group within the LGBTQ+ community so as to ensure they are represented in all forensic science, especially in research such as this where analytical chemistry is being used to determine the sex of the donor of a blood deposit. Unfortunately, there is a lack of trust in both law enforcement and the scientific community which was an insurmountable barrier to recruiting transgender blood donors. As an anonymous potential donor who ultimately refused participation stated, "because of the very real history of the criminalization of transgender and gender non-conforming people, the LGBTQ+ community has a healthy distrust of both law enforcement and the academy more generally, especially given how our experiences and other data have been used to undermine our own narratives and self-

understanding”. The research question regarding the effect of HRT on sex determination by Raman spectroscopy remains unanswered, however important discussions have begun and philosophical questions are being asked. For example, how can the concept of sex as a spectrum with a bimodal distribution be reconciled with current spectroscopic research & applications? [3] Is the binary output of male/female appropriate for spectroscopic determination of sex, or should this current model be revised to include the concept of sex as a spectrum? If sex is considered as a spectrum, what are the classes (e.g., male, female, intersex), and what are the match criteria and cut-off values? Last, how can scientists studying this recruit sample donors? Ultimately, the trust of the LGBTQ+ community needs to be gained back by the forensic science community in order for this research to be completed.

### **Works Cited**

- [1] Sikirzhyski, Vitali et al. Multidimensional Raman Spectroscopic Signatures as a Tool for Forensic Identification of Body Fluid Traces: A Review. Focal Point Review. 2011.
- [2] Sikirzhyskaya, Aliaksandra et al. Determining Gender by Raman Spectroscopy of a Bloodstain. Analytical Chemistry. 2017.
- [3] Montanez, A. (2017). Visualizing sex as a spectrum. Scientific American Blog, <https://blogs.scientificamerican.com/sa-visual/visualizing-sex-as-a-spectrum/>.