B32 The Development of RED-BLEU: A UV/Vis Assay Following Colorimetric Detection of EDTA

Sarah K. Schellhammer, BS, Richmond, VA 23220; Brittany C. Hudson, MS, Virginia Commonwealth University, Richmond, VA 23284; Alexandra M. Wright, BS, Richmond, VA 23225-6341; Catherine Cupples Connon, PhD, Virginia Commonwealth University, Richmond, VA 23284*

Learning Overview: After attending this presentation, attendees will be familiar with a new Ultraviolet/Visible (UV/Vis) spectroscopy component for the presumptive detection of disodium Ethylenediaminetetraacetic Acid (EDTA) in blood samples. This test is designed to accompany the previously reported colorimetric assay—Reverse EDTA Detection (RED)—with the main purposes of reducing subjectivity and false positives/negatives, as well as quantifying EDTA.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by supplementing previous research to bring a new presumptive method to the forefront that can indicate the presence of EDTA in blood samples, thereby aiding in the detection of planted blood evidence.

The detection of EDTA can help support or refute allegations of planted blood evidence because it is not a natural component of blood.[1] This research group has previously developed the RED colorimetric test for EDTA detection using Eriochrome® Black T (EBT), and this presentation will focus on the development of an extension of that method using UV/Vis to yield the Reverse EDTA Detection in Blood using EBT and UV/Vis (RED-BLEU) test.[2,3] To test this, a variety of samples with and without EDTA were subjected to the RED test, followed by analysis of 2µL of the tested sample on the NanoDrop™ 2000.

A multifaceted approach was taken to develop the UV/Vis portion of the assay: Wavelengths of interest were identified to corroborate the observed color change and EDTA quantification, the latter of which also required the development of a standard curve. To identify wavelengths of interest, samples prepared in triplicate concentrations of 0.1, 0.5, 1, and 10mg/mL EDTA were tested with the colorimetric assay, then processed in duplicate on the NanoDrop™. Absorbance values from these samples were compared to those of five blanks (processed in triplicate) to identify wavelengths with significant differences in absorbance. From this, wavelengths 192–196nm were identified as possible candidates for EDTA quantification, while 520–540nm, 640nm, and 650nm were noted as wavelengths of interest for an objective determination of color change.[4,5] Samples containing EDTA yielded a blue color change with a UV/Vis spectrum consisting of a discernable peak ~192–196nm and a flat baseline indistinguishable from blanks for the remainder of the spectrum. Conversely, samples without EDTA produced a pink color change with a UV/Vis spectrum exhibiting absorbance values elevated from the baseline ~520–540nm and less than the baseline at ~640nm and 650nm; however, they lacked the 192–196nm peak seen in samples with EDTA. Thus, the absorbance values at these wavelengths can be used to objectively determine whether EDTA is present.

The development of a standard curve to quantify the amount of EDTA present required multiple considerations: selection of wavelength, standard concentrations, and line of best fit, as well as identifying the impact (if any) of wait time. Wait time was assessed at two points: (1) 10, 30, or 60 minutes between adding the buffer and EBT indicator, followed by immediate UV/Vis testing; or (2) 10 or 30 minutes between the RED test and UV/Vis testing. Each criterion was tested in triplicate and was eliminated if the resulting standard curve had an R^2 <0.98. The best performing standard curve consisted of a linear trendline from absorbance values at 196nm and incorporated EDTA concentrations of 0.1, 0.5, 1, 3, 6, and 10mg/mL (processed in duplicate). Wait times of 10–30 minutes between addition of buffer and EBT indicator or 10 minutes between the RED test and UV/Vis analysis consistently yielded passing R^2 values.

Once validated, the RED-BLEU assay for EDTA detection will provide all forensic laboratories with a quick, inexpensive presumptive test to detect planted blood evidence.

Reference(s):

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2. Kim J., Vipulanandan C. Effect of pH, sulfate and sodium on the EDTA titration of calcium. *Cement and Concrete Research 2003*; 33(5):621-7.
3. Admin. BYJU'S. 2019, July 9. *Complexometric Titration–EDTA, Types of Complexometric Titration.*
4. Perkampus H.H. UV-Vis Spectroscopy and Its Applications. Berlin, Germany: *Springer Science and Business Media*, 2013.
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RED-BLEU, Blood, EDTA