Supplementary Materials for

Comparison of Colorimetric Analyses to Determine Cortisol in Human Sweat

Ethan Tu[a], Paul Pearlmutter[a], Michelle Tiangco[a], Gia Derose[b], Lina Begdache[b], Ahyeon Koh[a]*

[a] Department of Biomedical Engineering, Binghamton University-State University of New York, Binghamton, NY 13902

[b] Health and Wellness Studies Department, Binghamton University-State University of New York, Binghamton, NY 13902

[c] Department of Biomedical Engineering, Michigan State University, East Lansing, MI, 48824

* Corresponding Author: akoh@binghamton.edu; Tel +1 (607) 777-5422; Fax +1 (607) 777-5780

List of Materials

Analytical Assessments

Figures and Tables

Materials

Blue tetrazolium chloride, phenylhydrazine hydrochloride, and potassium hexacyanoferrate (III) were obtained from Alfa Aesar (Haverhill, MA). Isopropyl alcohol (99% ACS grade) was obtained from BDH Analytical Chemicals (London, England). Gold nanoparticles (15 nm diameter in aqueous solution, optical density= 1.0, concentration= 3 nM) were acquired from IMRA (Ann Arbor, MI). Ethanol (99% ACS grade) was purchased from Koptec (King of Prussia, PA). Butyl alcohol was bought from Macron Fine Chemicals (Allentown, PA). Iron (III) chloride and sulfuric acid (99% ACS grade) were obtained from Sigma Aldrich (Allentown, PA). Hydrocortisone acetate, isobutanol, and tetramethylammonium hydroxide (10% v/v) were acquired from TCI (Portland, OR). Methanol (99% ACS grade) and octanol (Lab grade) were purchased from VWR (Atlanta, GA). Artificial sweat and artificial saliva were purchased from Pickering Test Solutions (Mountain View, CA). Deionized water is purified (18.2 MΩ cm; total organic content <6 ppb) using a Millipore Milli-Q Gradient A-10 purification system (Bedford, MA).

Analytical Assessments

Unless otherwise specified, all experiments were conducted under room temperature (~23 ° C) in ambient conditions. Herein, we define dynamic range as the range of concentrations where the signals are directly proportional to the concentration of the analyte in the sample. Analytical limit of detection (LoD) is calculated by the equation $LoD=(3\times s_y)/b$, where s_y is the standard deviation of the blank signal and b is the slope of the calibration curve.[39] Experimental LoD is calculated as a function of the resolution of the spectrometer and the slope of the concentration curve. The Cary 60 UV-Vis spectrometer used in the study reads absorbance values in 1×10^{-4} increments. Therefore, experimental LoD is the smallest concentration change discernable by the spectrometer given increments of absorbance. The absorbance of each solution was measured in set intervals; therefore the reaction rates of presented methods were not explicitly determined.

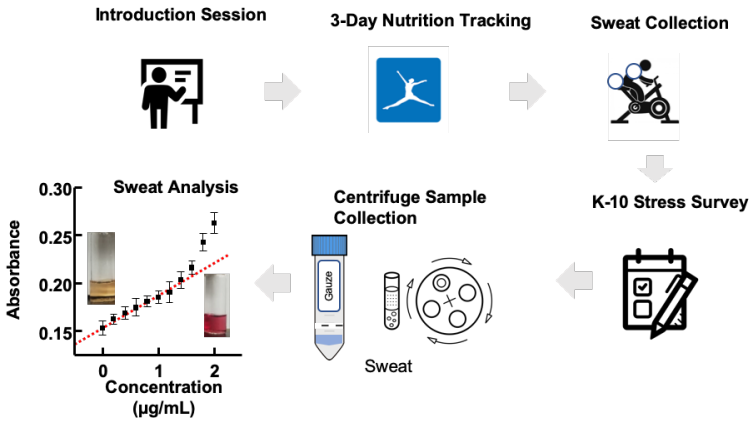

Figure S1. Schematic illustration of human sweat pilot study. Participants were asked to attend an introductory meeting to detail the study's design and purpose. Then, participants tracked their diet and water intake across three days using the Myfitnesspal app. On the fourth day, participants performed indoor cycling for 30–60 minutes at 8:00 am with sterilized gauze (3" × 12") attached to their armpits and lower back. Afterwards, the participants filled out the K10 survey. The gauze was then contained in a 50 mL centrifuge tube with a stopper placed midway. The sweat was centrifuged out of the gauze at 2500 rpm for 4 minutes. Finally, the cortisol concentration in the sweat was determined using the blue tetrazolium reagent.

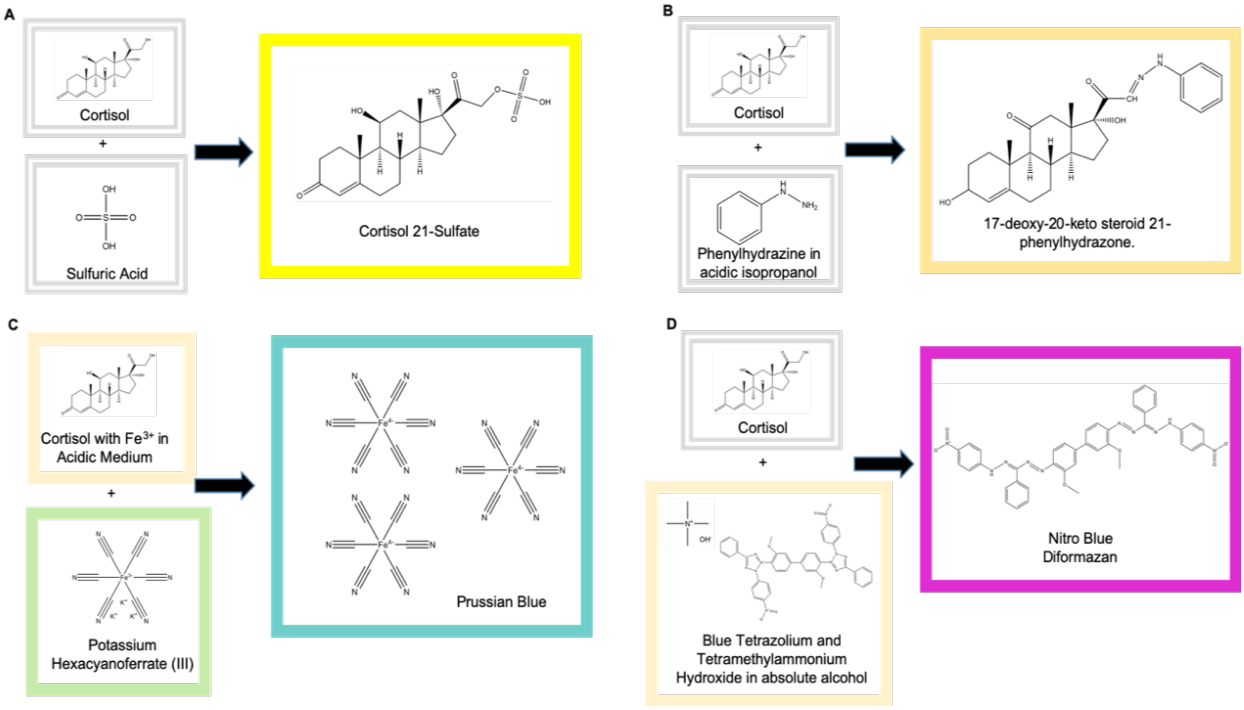

Figure S2. Colorimetric Reactions of Different Reagents with Cortisol. Outlined colors indicate color of the solution. (A) Sulfuric acid complexes with cortisol to form cortisol 21-sulfate. (B) The classic Porter-Silber reagent consists of phenylhydrazine in acidic alcohol. This reagent complexes with cortisol to form cortisol 21-phenylhydrazone. (C) Cortisol reduces iron (III) to iron (II) in an acidic medium. The iron (II) then complexes with potassium hexacyanoferrate (III) to form Prussian blue. (D) Blue tetrazolium oxidizes the C-17 side chain of cortisol, which is then reduced by tetramethylammonium hydroxide to for diformazan

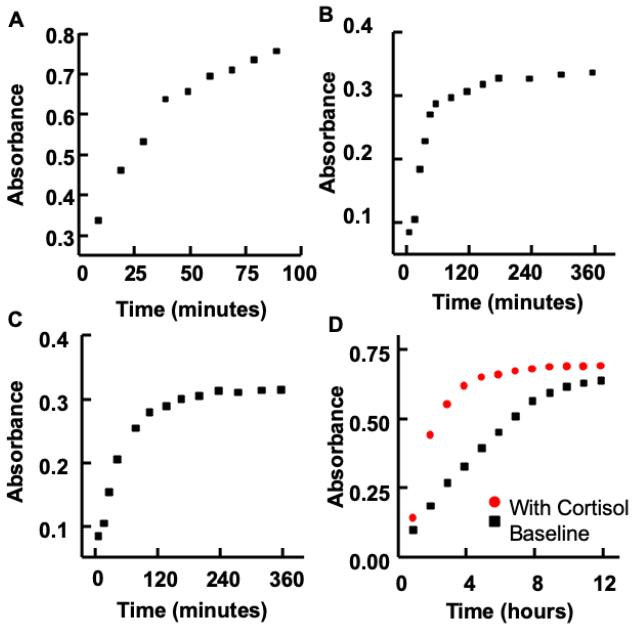

Figure S3. Time to full color development of various reagents. (A) Sulfuric acid absorbance will increase until sample is unviable. (B) AuNP Porter absorbance will increase until it plateaus at around 90 min. (C) Prussian blue absorbance will increase until it plateaus around 100 min. (D) Blue tetrazolium absorbance is reported with a baseline solution and cortisol solution. Baseline solution will also reach the same absorbance as solutions with cortisol but at a much slower rate. After 12 hours, all solutions are near indistinguishable.

Table S1. Porter-Silber reaction with various alcohol solvents.

Solvent system	***Time to Initial Color Development (minutes)***	***Time to Full Color Development (minutes)***	***Time to Initial Color Development (minutes)***	***Time to Full Color Development (minutes)***	***Characteristic Absorbance Peaks (nm)***	***Remarks***
Temperature	***23 °C***	***23 °C***	***70 °C***	***70 °C***		
Methanol	150	405	75	150	410	None
Ethanol	120	360	60	135	410	Normal Porter-Silber Reagent
Butanol	105	225	60	105	410	Slightly more viscous
Octanol	90	180	45	105	410	Creates stratum, color appears in upper stratum
Isopropanol	90	150	45	90	410	None
Isobutanol	90	150	40	90	410	Creates stratum, color appears in upper stratum
Isopropanol with AuNP	60	120	30	80	410, 560	Additional peak may arise due to AuNP

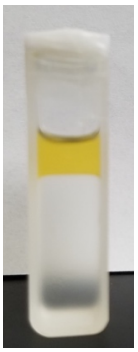

Figure S4. Porter-Silber reagent with octanol. At higher carbon content, alcohols become immiscible with water. This creates a stratum within the reagent, where color development only occurs within the alcohol. Additionally, the overall solution becomes cloudy and opaque.

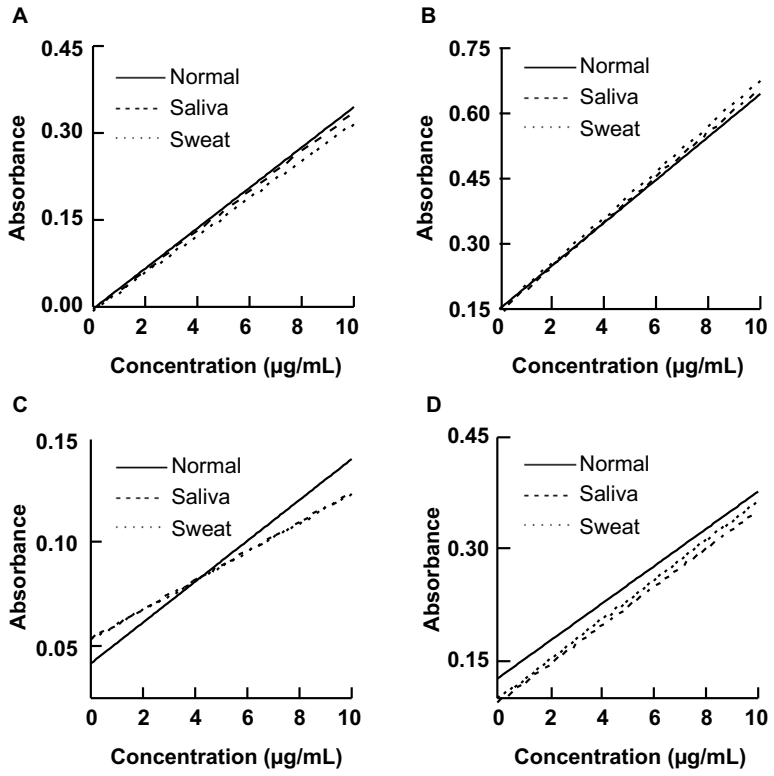

Figure S5. Comparison of concentration curves derived from cortisol stock, artificial saliva, and artificial sweat. Calibration curves of **(A)** AuNP Porter-Silber **(B)** Blue Tetrazolium **(C)** Prussian Blue **(D)** Pure Sulfuric Acid that are constructed with various matrix including water, artificial saliva, and artificial sweat. All curves are nearly identical confirming that no interferences or biomarkers would have an interaction with the methods.

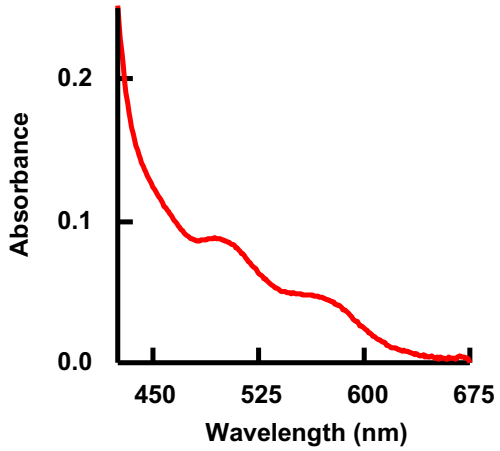

Figure S6. Representative absorbance spectrum of human sweat in blue tetrazolium reagent. An absorbance value of 0.095 at 510 nm corresponds to 162 µg/mL of cortisol in sweat.