SUPPORTING INFORMATION

Machine learning-based quantification of (-)-trans-delta-tetrahydrocannabinol from human saliva samples on a smartphone-based paper microfluidic platform

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Figure S2. The channel design and dimensions. The test zone (yellow), labeled with two lines, is located closer to the sample entrance area (blue). The sample flows from the entrance area, pass through the test area, and finally to the far right, via capillary action.

Figure S3. Comparisons of raw and processed images over different brands of smartphones.

Figure S4. Smartphone-based fluorescence microscopic images of paper microfluidic chips (pre-loaded with THC-BSA) before and after loading antibody-conjugated nanoparticles. Two different light sources were used – 460 nm LED and a white LED.

Figure S5. The size distributions of bare vs. antibody-conjugated nanoparticles on paper microfluidic chips, as imaged by a smartphone-based fluorescence microscope. Diameters (in arbitrary units) were determined using ImageJ. In all four cases, the diameters were not substantially different. This result indicates that the particles do not self-aggregate by antibody conjugation and do not aggregate by the positive sample presence.

Figure S6. Durability test of the paper chips, pre-loaded with THC-BSA and antibody-nanoparticles and stored at room temperature and 4°C. Assays were conducted using a smartphone-based fluorescence microscope with 0, 3, and 10 ng/mL THC dissolved in DI water. No significant changes were observed over time (up to 10 days) for all assays, except the 0 ng/mL (negative sample) results after day 5. In all cases, the negative assay results differed significantly (p < 0.05) from the positive ones.

Figure S7. Characteristics of saline- and DI-water diluted human saliva samples. (A) Sensor outputs (nanoparticle counts in the test zone) against THC concentrations from 12 saliva samples. (B) The recovery ratio of negative saliva controls in THC detection. Sensor outputs were normalized to those with DI water were plotted over 12 saliva samples, varying dilutions. Dilutions were made with 0.9% w/v saline. (C) Transmittance (%), (D) density, (E) viscosity, and (F) surface tension (as measured by the pendant droplet method) are shown for 12 saliva samples with varying dilutions.

Figure S8. Correlation analysis between the saliva samples’ physical properties and recovery: (A) transmittance, (B) density, (C) viscosity, and (D) surface tension.

Figure S9. ML prediction of THC concentration. k-NN was shown as an example. Three pictures were taken from each lane and analyzed through ImageJ to obtain the pixel numbers of captured nanoparticles in the test zone. A single saliva samples were diluted at four different dilutions and pipette-added to four lanes of a single chip. Four-dimensional data, a, b, c, and d, were obtained and fed into the ML model, using the training database.

Figure S10. Workflow of ML-based THC quantification. First level: prediction among 0, 100, and 300 pg/mL using 10% and 1% dilution sets and dismissing 0.1% and 0.01%. If 0 or 100, stops. If 300, move to the second level prediction among 0, 1000, and 3000 pg/mL. If 0, finalize at 300. If 1000, finalize at 1000. If 3000, move to the third level prediction among 0, 10000, and 30000 pg/mL.

Table S1. An overview of recently reported methods and commercial kits for THC detection.
SUPPLEMENTARY EXPERIMENTAL SECTION

Reagents and samples. THC-BSA (17.03 mg/mL in PBS buffer, pH at 7.4) was purchased from Fitzgerald Industries International (80-IT62; Fitzgerald Industries International, Acton, MA, USA), where BSA (bovine serum albumin) was conjugated to THC as a hapten. THC was additionally purchased from Sigma-Aldrich (T-047; Sigma-Aldrich, Inc., St. Louis, MO, USA). The CBD sample was purchased from Cayman Chemical (90080; Cayman Chemical, Ann Arbor, Michigan, USA). All human saliva samples were purchased from Innovative Research (IR100044SD; Innovative Research, Inc., Novi, MI, USA). For each saliva sample, a 10%, 1%, 0.1%, and 0.01% dilution sets were made with 9% w/v NaCl solution (saline solution) separately. 500-nm diameter yellow-green carboxylated polystyrene nanoparticles were purchased from Magsphere (CAF-005UM; Magsphere, Inc., Pasadena, CA, USA), 2.5% w/v in stock. The manufacturer reported the peak excitation wavelength of 488 nm and the peak emission at 509 nm. The CN95 nitrocellulose paper was purchased from Sartorius AG (Goettingen, Germany). Unless specified otherwise, the remaining reagents were purchased from Fisher Scientific (Pittsburgh, PA, USA) or Sigma (St. Louis, MO, USA).

Density of saliva samples. The density for each saliva dilution was measured by first zeroing out an Ohaus Adventurer electronic balance (Uline, WI, USA) with a PCR test tube. Once the balance has been zeroed, 100 μL of a saliva dilution was pipetted into the PCR test tube to be weighted in milligrams. Using the known weight and volume, the density of the saliva dilution can be calculated using the equation \( \rho = \frac{m}{V} \). This procedure was then repeated two more times for the same saliva dilution. The three densities were then averaged and analyzed to determine any significant differences between saliva dilutions. In addition, the density of DI water was also measured to compare with the densities of the saliva dilutions.

Surface tension of saliva samples. The surface tension of the different saliva dilutions was measured by using ImageJ to analyze the images of the droplets of the saliva dilutions hanging at the end of a blunt-end needle through a syringe (pendant droplet method). Images were taken at 6 time points after a droplet was pushed out of a fixed hydrophobic syringe needle with a smartphone. The 6 different time points was at 0, 2, 4, 6, 8, and 10 min. For better clarity, the droplet was illuminated by a halogen lamp.

Turbidity of saliva samples. Using a spectrometer (Ocean Insight, Orlando, FL, USA) and Ocean View software (Ocean Insight, Orlando, FL, USA), the intensity values at 730 nm and 860 nm were measured for DI water and each saliva dilution. For each sample, three repeats were performed in total. Using the intensity values for DI water and the saliva dilution, the transmittance and absorbance was calculated for each repeat. Transmittance (%) was calculated using the equation \( T = 100 \times \frac{I_0}{I} \) where \( I_0 \) represents the intensity value for DI water and \( I \) represents the intensity value for the sample being tested. Using the same variables, absorbance was calculated using the equation \( A = \log_{10}(\frac{I_0}{I}) \).

Viscosity of saliva samples. Using the 1831 Ostwald U-Tube viscometer (YuchengTech, Zhejiang, China) with a diameter of 0.55 mm, DI water and saliva dilutions were measured for their kinetic viscosity. The test was performed by first pouring enough of the sample being tested until it reaches the labeled marker on the large bulb of the viscometer. The sample was then vacuumed above the labeled tick on the smaller end and released to measure the kinetic viscosity. For accuracy, the experiment was recorded using a smartphone and analyzed in slow motion to determine the time it takes for the sample to pass the designated zone marked by two ticks on the smaller end. This procedure was repeated 5 times into total for DI water and each saliva dilutions. The viscosity of the various saliva dilutions (subscript \( Y \)) was calculated using the equation \( \eta_Y = \eta_{water} \left(\frac{\rho_Y I_Y}{\rho_{water} I_{water}}\right) \) where \( \rho \) represents density and \( t \) represents the average time it took for the sample to pass the designated region on the smaller end of the viscometer.

Durability test of the prepared paper chips. A durability test of the paper chips was conducted by storing them at either room temperature or 4°C for up to 10 days. The paper chips were pre-loaded with THC-BSA, followed with antibody-nanoparticles, and air-dried. They were packaged in foil pouches and stored at room temperature and ambient humidity or 4°C in a refrigerator. The tests used THC solutions of 0 ng/mL (negative control), 3 ng/mL and 10 ng/mL (two positive controls). Assays were conducted in the same manner described in the Experimental section, using the smartphone-based fluorescence microscope.
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**Journal publications**

| Sample type | Sample volume | Assay time | Cost       | Sensing element       | Sensing principle         | LOD          | DOI link                  |
|-------------|---------------|------------|------------|-----------------------|---------------------------|--------------|--------------------------|
| Saliva      | 10 μL         | 5 min      | Not available | OECT                 | Oxidation reaction        | 1 nM         | 10.1039/d0tb02951e17     |
| Saliva      | 100 μL        | 1 min + incubation | Not available | EIS                  | Impedimetric measurement  | 0.1 ng/mL    | 10.1038/s41598-019-49185-y18 |
| Saliva      | 15 μL         | 30 s       | Not available | Screen-printed carbon electrode | Chronoamperometric reduction | 25-50 ng/mL | 10.1186/s13065-016-0148-19 |
| Saliva      | 150 μL        | 10 min     | $7,000 for IR camera | LFIA   | Thermo-photic imaging | 2 ng/mL     | 10.1364/BOE.3889905     |
| Saliva      | 150 μL        | 20 min     | Not available | Smartphone with IR filter | LFIA   | 2 ng/mL     | 10.1039/D0A N01850C16     |

OCET = organic electrochemical transistor; EIS = electrochemical impedance spectroscopy; LFIA = lateral flow immunochromatographic assay

**Commercial kits**

| Sample type | Sample volume | Assay time | Cost   | Sensing element       | Detection principle | LOD          | Manufacturer          |
|-------------|---------------|------------|--------|-----------------------|---------------------|--------------|----------------------|
| Urine       | Not mentioned | 5-30 min   | $10    | Gold nanoparticles    | LFIA                | 15 ng/mL     | Ütest                |
| Urine       | Not mentioned | 10 min     | $10    | Gold nanoparticles    | LFIA                | 18 ng/mL     | NarcoCheck           |
| Saliva      | Not mentioned | 10 min     | $5     | Gold nanoparticles    | LFIA                | 20 ng/mL     | Nicotests            |
| Saliva      | Not mentioned | 10-12 min  | $10    | Gold nanoparticles    | LFIA                | 10 ng/mL     | NarcoCheck           |
| Saliva      | Not mentioned | 5 min      | $100-$500 | Mobile test system | Cartridge analysis  | 25 ng/mL     | Abbott               |