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Supporting Information

An origami paper-based analytical device for rapid detection of testosterone in healthcare food

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Contents

Chemicals and materials

Testosterone (TES), Monoclonal testosterone antibody (TAb), HRP-labeled testosterone (HRP-TES), acrylamide (AM), methyltestosterone (MT), boldenone (Bold) and trenbolone (Tren) were obtained from Wuxi Determine Biotechnology Co., LTD. (Wuxi, China). 3,3',5,5'-tetramethybenzidine (TMB) and bovine serum albumin (BSA) were purchased from Beijing Solarbio Science and Technology Co., Ltd.(Beijing, China). Tween 20 was provided by Thermo Fisher Scientific (Toronto, ON, Canada). Pullulan and 1× PBS solution (pH 7.4) was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Nitrocellulose membrane with backing (HF 120 and HF 180) and cellulose membrane (Grade 1 and Grade 5) were from GE Healthcare (Chicago, IL, USA). Washing buffer was made from 1× PBS containing 0.05% (v/v) Tween 20. Blocking buffer consisted of 0.05% (v/v) Tween 20 and 3% (w/v) BSA in 1× PBS. Color buffer (1× PBS) contained 53.33 mM HAc-NaAc buffer with 2.67 mM TMB and 3.33 mM H_2O_2, pH 4.5.

Fabrication of oPAD

The proposed oPAD is comprised of two units, shown as Zone 1 and Zone 2 (Scheme 1). We first printed outlines of Zone 1 and Zone 2 along with a connecting channel, on a nitrocellulose membrane using a wax printer (Xerox ColorQube 8570N), followed by heating at 120 °C for 2 min to form hydrophobic barriers. These two units were assembled using adhesive tape, and produced a sensor with the circular zone (Zone 1 and Zone 2) of diameter 0.5 cm and a connecting bridge 1 cm long and 0.2 cm wide. 8 μL of TAb was added to Zone 1 in the form of "open bridge" and incubated for 15 min before washing twice by adding 20 μL of washing buffer. The blocking buffer of 20 μL was then added to Zone 1 and incubated at room temperature for 40 min. After washing twice by 20 μL of washing buffer, 6 μL of 10% pullulan (w/v) solution was then inkjet-printed onto Zone 1 and the oPAD was dried and stored at 4 °C.

TES detection

8 μL of TES and 8 μL HRP-TES were mixed and added to Zone 1. After a reaction of 30 min, Zone 1 was unfolded to connect with Zone 2. The unbound HRP-TES flowed to Zone 2 by adding 10 μL washing buffer for four times via the "connected bridge". After Zone 1 and Zone 2 were dried, 10 μL of color buffer containing TMB and H_2O_2 was added. The colorimetric reaction finished within 1 min, and captured images were transferred to a computer for further analysis by Image J. For the control group, TES was not added into Zone 1 after blocking, and the other steps were the same as the experimental group. All experiments were independently conducted at least three times.

Sensitivity, selectivity and real sample test

To assess the detection limits, 8 μL of various concentrations of TES (0.01, 0.1, 1, 10, 100, 1000, 5000, and 10000 μg/L) were added to Zone 1 and assays were performed as described above. As for selectivity analysis, 8 μL of 50 μg/L acrylamide (AM), methyltestosterone (MT), boldenone (Bold), trenbolone (Tren) and TES were introduced to perform the experiments as described above.

To evaluate the performance of this integrated oPAD in real samples, we investigated the recovery of TES using spiked healthcare food such as tablets and oral liquids, which were purchased from a local pharmacy and was kept at 4 °C. The samples were first confirmed to be free of TES using HPLC-MS/MS (Thermo TSQ Quantum Ultra; Thermo Fisher Scientific, Toronto, ON, Canada). 0.1 g of the tablet was ground into powder and dissolved in 1× PBS of 1 mL, obtained solution was filtered through a 0.22 μm nylon membrane filter. The oral liquid samples were 10-fold diluted using 1× PBS. Then, the tested samples were individually spiked with 10, 50 and 100 μg/L of TES, with six parallel samples prepared for each concentration.

Artificial urine sample

Artificial urine sample consisted of 170 mmol/L urea, 90 mmol/L sodium chloride, 25 mmol/L ammonium chloride, 25 mmol/L sodium carbonate, 10 mmol/L sodium sulfate, 7 mmol/L potassium dihydrogen phosphate, 7 mmol/L dipotassium hydrogen phosphate, 2.5 mmol/L calcium chloride, 2 mmol/L magnesium sulfate, 2 mmol/L citric acid, 1.1 mmol/L lactic acid, and 0.4 mmol/L uric acid dissolved in distilled water. This sample was then adjusted to pH 6 with 1 mol/L hydrochloric acid[1].

References

1. I. Lewinska, K. Kurdzialek and L. Tymecki, *Molecules*, 2021, **26**, 6282.