Electronic Supporting Information

Non-Invasive Iron Deficiency Anemia Diagnosis: A Saliva-Based Approach Using Capillary Flow Microfluidics

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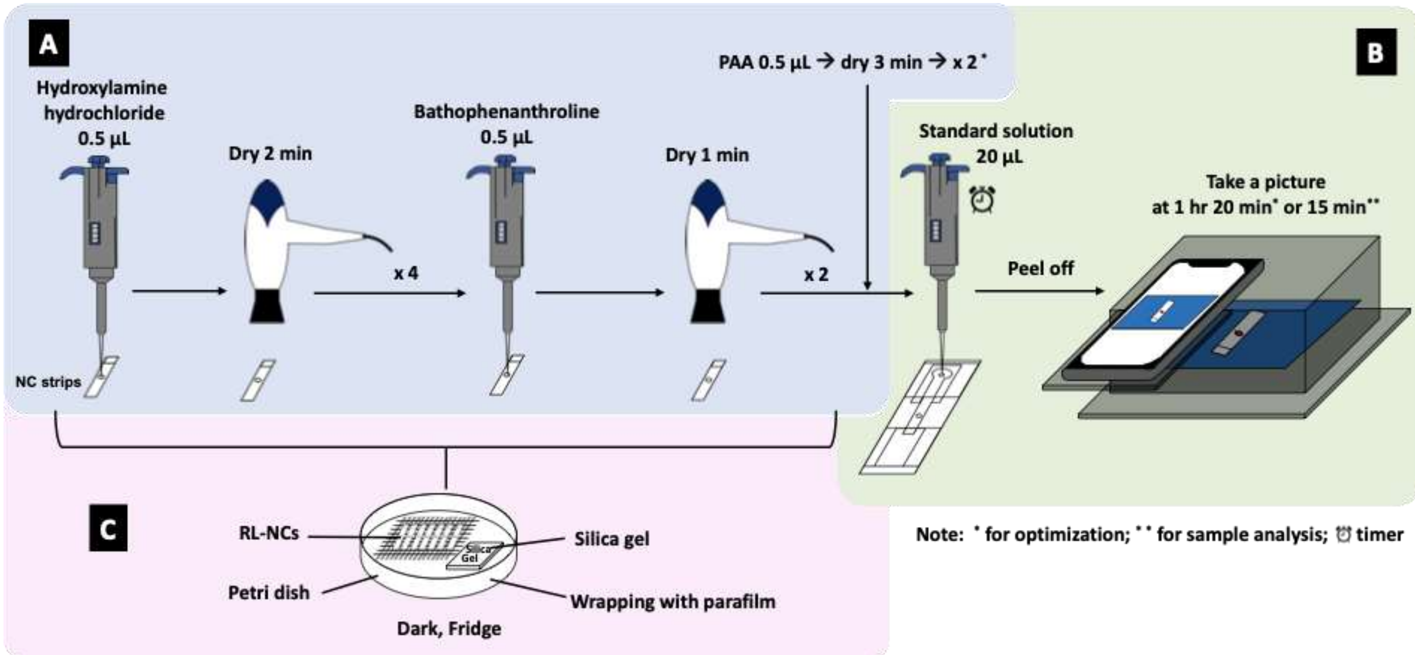
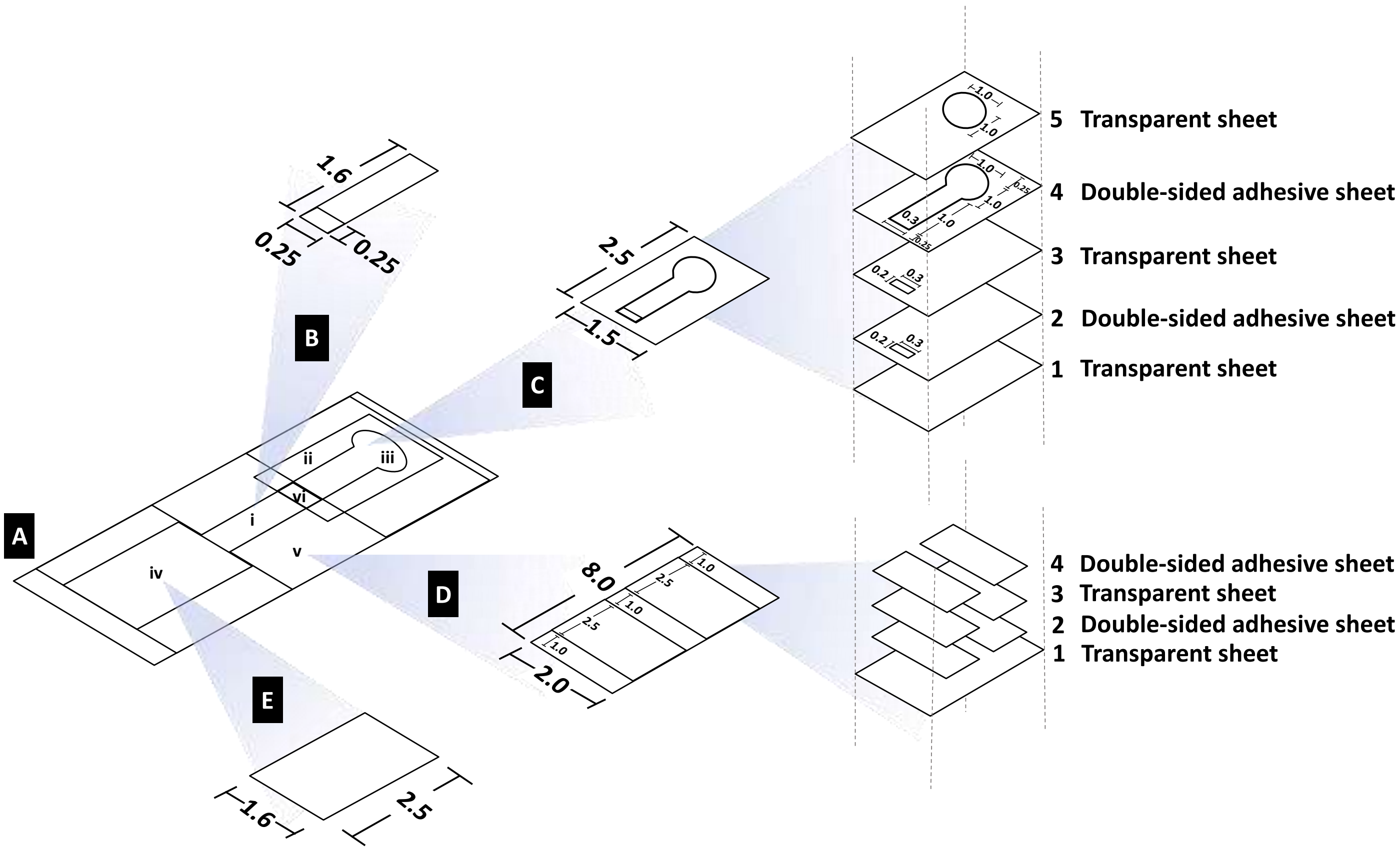


Scheme S1. Analytical operation in this work **(A)** RL-NCs preparation **(B)** Procedure of Fe^{2+} detection **(C)** RL-NCs storage in a suitable condition before Fe^{2+} detection.



i Reagents loaded NC strips (RL-NCs)
ii Half strip device (HSD)
iii Sample loading inlet
iv Waste pad
v Double sided adhesive (DSA)
vi NC channel

Scheme S2. (A) The component of the capillary flow-driven microfluidic device consists of four sections as follows: **(B)** 0.25 x 1.6 cm reagents-loaded nitrocellulose strip (RL-NC) with a line at 0.25 cm from the bottom, **(C)** 1.5 x 2.5 cm half strip device with five layers illustrated above **(D)** 2.0 x 8.0 cm double-sided adhesive sheets with four layers illustrated above and **(E)** 1.6 x 2.5 cm waste pad.

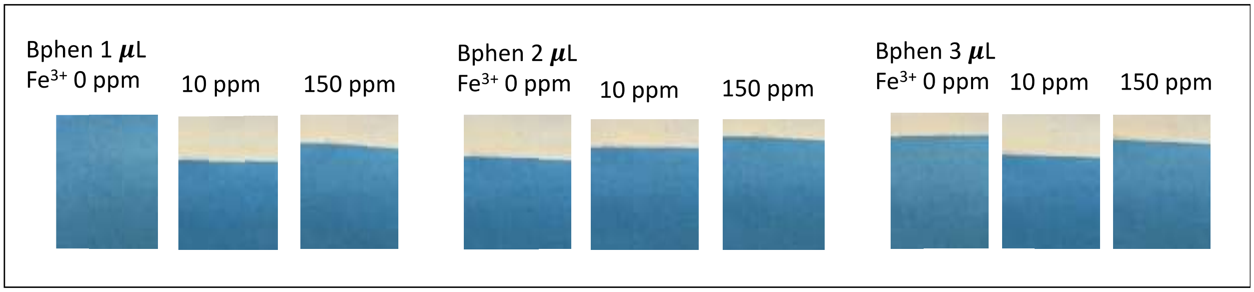


Figure S1. Optimization of Bphen volume

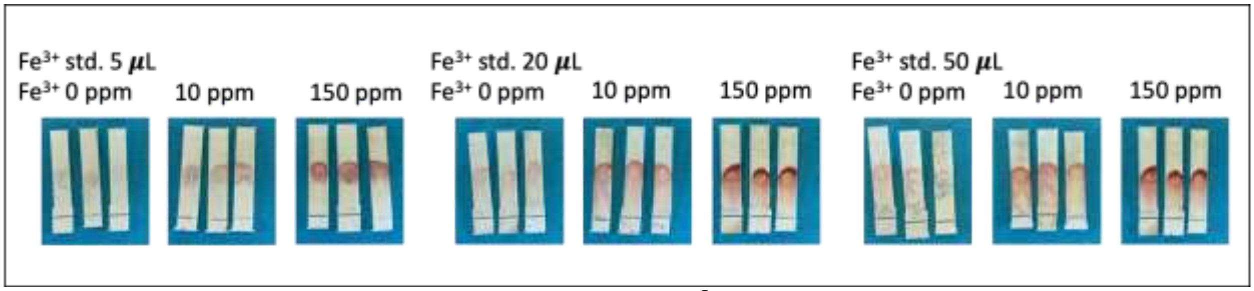


Figure S2. Optimization of Fe^{3+} standard volume

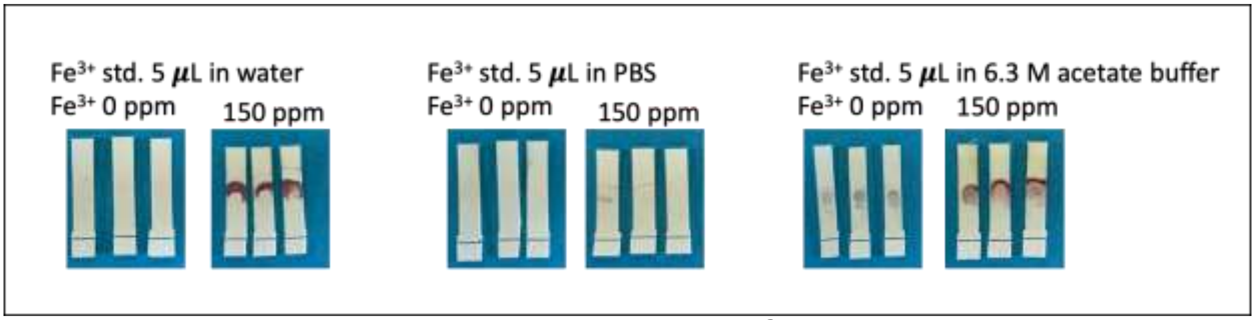


Figure S3. Optimization of solvent of Fe^{3+} standard solution

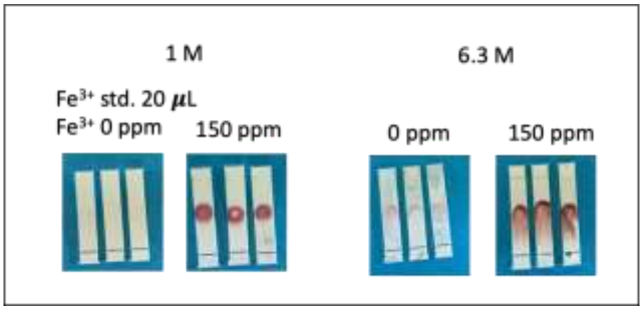


Figure S4. Optimization of acetate buffer concentration

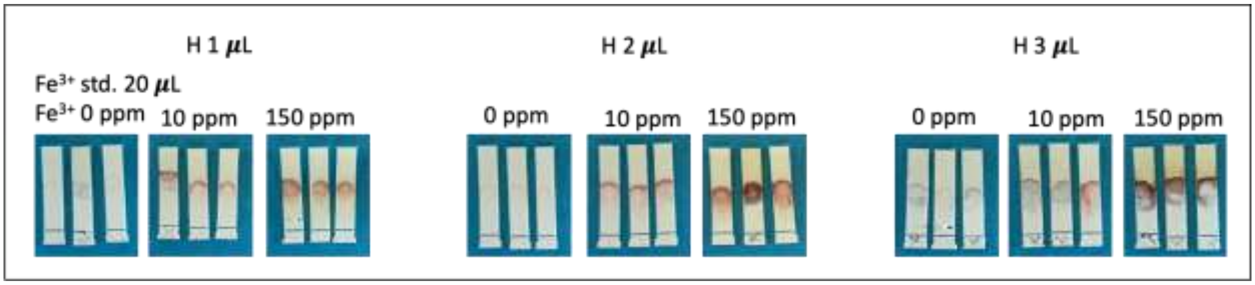


Figure S5. Optimization of hydroxylamine hydrochloride volume

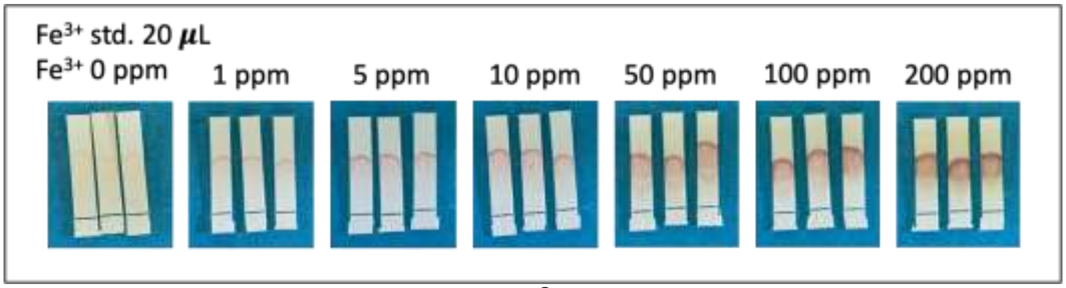


Figure S6. Calibration curve at Fe^{3+} standard solution 0 to 200 ppm

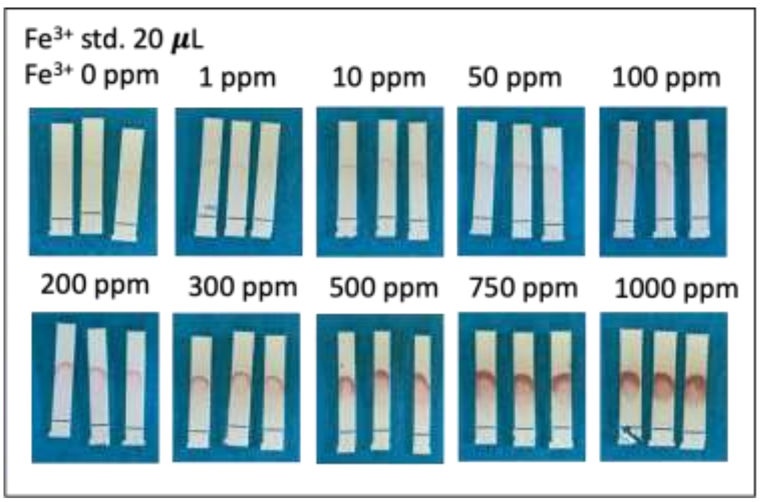


Figure S7. Calibration curve of pooled saliva

Procedure

S1.) Device fabrication and design

The fluidic devices were designed using Onshape, a computer-aided design (CAD) software system (PTC, MA, USA), and cut using a CO_2 laser cutter (Zing 10000, Epilog Laser) to pattern layers of transparency films (9984) and double-sided adhesive sheets. The Half Strip Device

(HSD) consists of three layers of transparent sheets and two double-sided adhesive sheets (**Scheme S1)**.

For the 1st layer, the transparent sheet was cut as rectangular (1.5 x 2.5 cm). This layer served as the base of half strip device. After that, the 2nd and 3rd layers were assembled with the double-sided adhesive and transparent sheets, respectively. These layers had a (0.3 x 0.2 cm) rectangular cavity higher up 0.25 cm from the bottom side of the half strip device to be a channel for inserting a RL-NCs (called an NC (nitrocellulose) channel, shown in **Scheme. S2 (A, vi)**. On the 4th layer, a cavity was created as a connection shape between the circle (d = 1.0 cm) and rectangular (0.3 x 1.0 cm) by using the double-sided adhesive sheet. As a result, the 5th layer was built the same size as the circle cavity for loading the sample using the transparent sheet. All these layers were assembled layer by layer and continuously compressed with bench top manual presses (Caver 4122, Caver, Inc., IN, USA) at room temperature to thoroughly combine all layers. This combined shape was used for the solution flow from a sample loading inlet (**Scheme. 2S (A, iii)**) to the RL-NCs. A waste pad (**Scheme. S2 (A, iv)**) contained the whole solution after flowing through the RL-NCs. This cellulose fiber sample pad (CFSP223000, EMD Millipore Corporation, MA, USA) was cut as a (1.6 x 2.5 cm) rectangular shape.

A double-sided adhesive sheet (**Scheme. S2 (A, v)**) was fabricated to hold the half-strip device, RL-NCs, and waste pad together. The adhesive parts were manufactured by assembling a layer of (2.0 x 2.5 cm) transparent sheet and two layers of (2.0 x 2.5 cm) double-sided adhesive sheet (468 MP, 3M Corporation, MN, USA). The double-sided adhesive sheets sandwiched the transparent sheet on both sides. Two pieces of the double-sided adhesive sheet parts were stuck 1.0 cm apart on a (2.0 x 8.0 cm) transparent sheet, which served as the base of the double- sided adhesive tape sheet, as shown in **Scheme. S2 (D)**.

S2.) Device assembly

All the steps for assembling the device were represented in **Scheme. S2 in SI**. The Fe^{2+} detection can be operated by setting up all sections of the capillary-flow device together, starting with inserting a head side of RL-NCs into the NC channel of the strip device (**Scheme. S2 (A, vi) in SI**). The half-strip device was subsequently stuck on double-sided adhesive sheets by attaching the NC channel past a double-sided adhesive part about 0.2 cm to avoid solution leaking. The tail side of RL-NCs was finally covered with a waste pad about 0.2 cm past the line on RL-NCs.

Reaction

$$2NH_2OH_{(aq)} + 4Fe^{3+}_{(aq)} \rightarrow N_2O_{(g)} + 4Fe^{2+}_{(aq)} + H_2O_{(l)} + 4H^{+}$$

$$Fe^{2+} + 3Bphen \rightarrow ([Fe(Bphen)_3]^{2+})$$