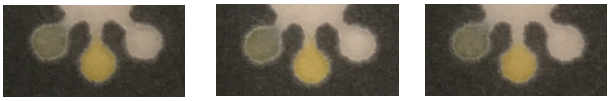
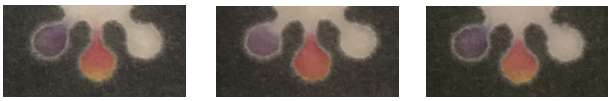
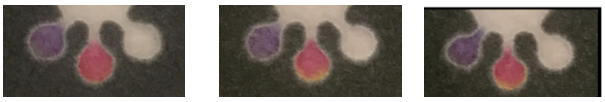
# Supplementary Data

Table S1. Viscosity Measurement

| % SCMC | Shear rate,1/s | True Visc, mPa-s | | | Mean | SD |
|---|---|---|---|---|---|---|
| 0.25% | 7000 | 1.53 | 1.538 | 1.541 | 1.54 | 0.0035 |
| 0.50% | 6000 | 1.871 | 1.866 | 1.872 | 1.87 | 0.0032 |
| 0.75% | 4000 | 2.174 | 2.181 | 2.178 | 2.18 | 0.0035 |
| 1.00% | 4000 | 2.721 | 2.734 | 2.721 | 2.73 | 0.0075 |
| 1.25% | 3000 | 4.181 | 4.169 | 4.165 | 4.17 | 0.0083 |
| 1.50% | 2000 | 5.036 | 5.199 | 5.054 | 5.10 | 0.0894 |

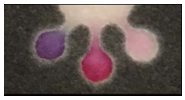
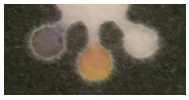
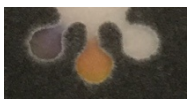
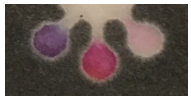
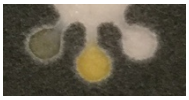
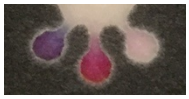
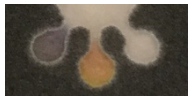
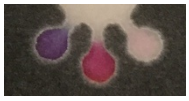
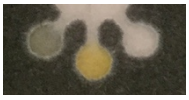
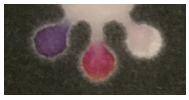
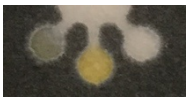
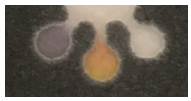
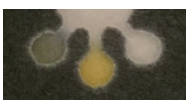
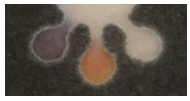
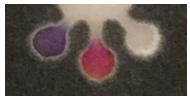
SCMC: sodium carboxymethylcellulose, Visc: Viscosity

Table S2. The pH color change on proposed PADs

| No. | %SCMC | Color change on PADs | | | pH on PADs | pH meter |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | |
| 1 | 0.25 |  | | | 6.6-6.8 | 6.401 ± 0.018 |
| 2 | 0.25 |  | | | 8.0 | 8.003 ± 0.002 |
| 3 | 0.50 |  | | | 7.2-7.4 | 7.284 ± 0.037 |
| 4 | 0.50 |  | | | 6.8-7.0 | 6.858 ± 0.002 |
| 5 | 0.75 |  | | | 5.8 | 5.700 ± 0.060 |
| 6 | 0.75 |  | | | 7.8 – 8.0 | 7.826 ± 0.045 |
| 7 | 1.00 |  | | | 7.8 – 8.0 | 8.252 ± 0.001 |
| 8 | 1.00 |  | | | 7.0 | 7.199 ± 0.051 |
| 9 | 1.25 |  | | | 7.0 | 7.107 ± 0.019 |
| 10 | 1.25 |  | | | 8.5 | 8.908 ± 0.049 |
| 11 | 1.50 |  | | | 6.6 | 6.552 ± 0.021 |
| 12 | 1.50 |  | | | 7.8 | 7.896 ± 0.010 |

SCMC: sodium carboxymethylcellulose, PADs: paper-based analytical devices

Table S3. Reagent stability

| Days | Spiked concentration (mg/dL) | Condition | PADs (mg/dL) (n=6) | pH on PADs | | |
|---|---|---|---|---|---|---|
| | | | | Low (5.651) | Normal (7.132) | High (10.186) |
| 2 | 1.00 | RT | 0.94 ± 0.23 |  |  |  |
| | | 4 ◦C | 0.90 ± 0.13 |  |  |  |
| 4 | 1.00 | RT | 0.81 ± 0.12 |  |  |  |
| | | 4 ◦C | 0.96 ± 0.19 |  |  |  |
| 7 | 1.00 | RT | 0.99 ± 0.09 |  |  |  |
| | | 4 ◦C | 1.02 ± 0.10 |  |  |  |
| 10 | 1.00 | RT | 0.97 ± 0.13 |  |  |  |
| | | 4 ◦C | 1.07 ± 0.17 |  |  |  |
| 14 | 1.00 | RT | 0.68 ± 0.15 |  |  |  |
| | | 4 ◦C | 1.07 ± 0.13 |  |  |  |
| 21 | 1.00 | RT | 0.72 ± 0.18 |  |  |  |
| | | 4 ◦C | 1.03 ± 0.31 |  |  |  |
| 30 | 1.00 | RT | 0.92 ± 0.20 |  |  |  |
| | | 4 ◦C | 1.04 ± 0.08 |  |  |  |

Day 30 Spectrophotometer Nitrite concentration 1.01 ± 0.01 mg/dL

1. pH color index preparation

For preparation the pH color scale, The PADs design with diameter 4 mm of detection area was fabricated using wax printing technique and 0.3 µL of each indicator was deposited in to pH sensing area. Phenol red indicator was required 0.6 µL or 2 drops of solution. The PADs were allowed to dry at room temperature for 5 min. The scale for reading of pH was conducted as follows: 0.5 µL of buffer solution or saliva sample containing a various of pH in range 5 to 10 were added on top of sensing area. The change of color on PADs was recorded within 1-2 min via smartphone.

2. Optimization the optimum types and pH of washing buffer

To investigate the effect of pH on the formation of azo dye complex, citrate buffer with pH 5, Na-phosphate buffer with pH 7.2, borate buffer with pH 10 and distilled water were prepared and tested on proposed device. In this study, 0.7 µL of Griess reagent was immobilized into detection areas and control area and left to dry in the dark plate for 10 min. Then,13 µL of saliva specimen containing 0.5 or 1.0 mg/dL of nitrite was added into sample area. The fluid can be directly absorbed into sample area and flowed through the channel. Finally, each buffer was applied into sample area and waiting until saliva fluid was spread out into detection areas, approximately 5-7 min. Figure S1 represents the color signal of each buffer and the photographs of PADs after adding Na-phosphate buffer.

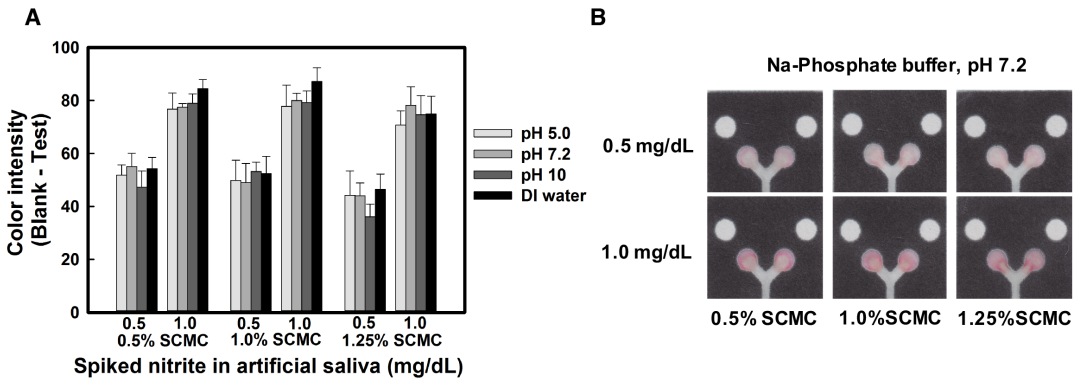



Figure S1. (A) Represent the color intensity of azo dye complex after adding with citrate buffer, Na-phosphate buffer, borate buffer and distilled water (n=4) (B) Photographs of PADs with nitrite concentration 0.5 mg/dL and 1 mg/dL in various viscosity of saliva specimen

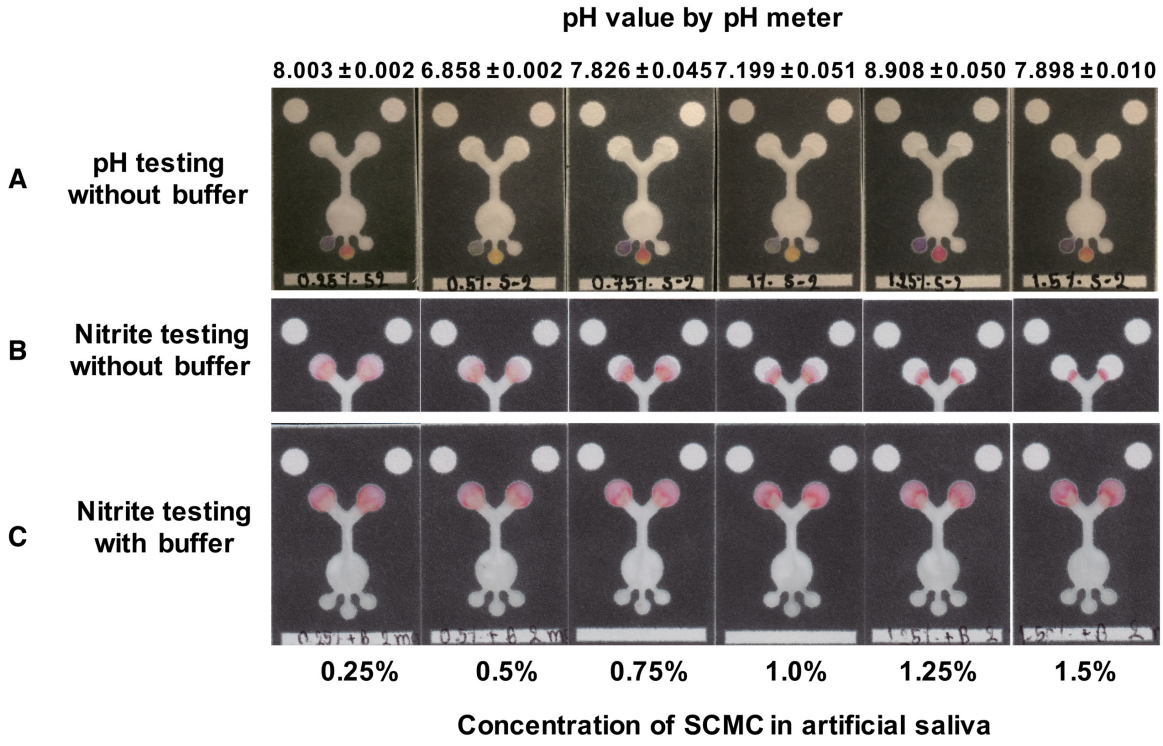



Figure S2. (A) The pH testing on proposed PADs was not affected by the viscosity of saliva. (B-C) Nitrite measurement was effected by the viscosity of solution and the accuracy for nitrite quantification was improved by added the washing buffer to spread out saliva solution.

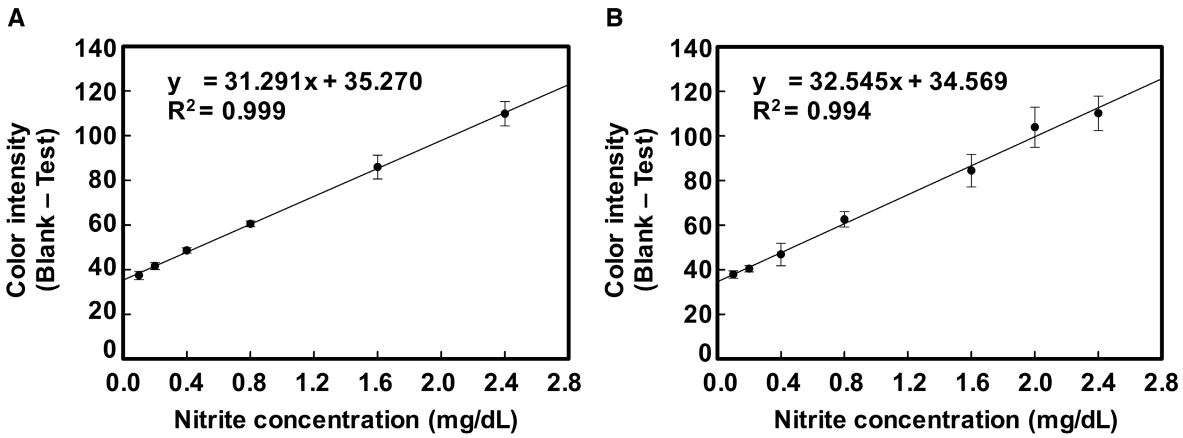



Figure S3. (A) Show analytical curve for nitrite measurement from artificial saliva containing 0.50% SCMC (n=6) (B) Show data from 1.50% SCMC in artificial saliva (n=6)

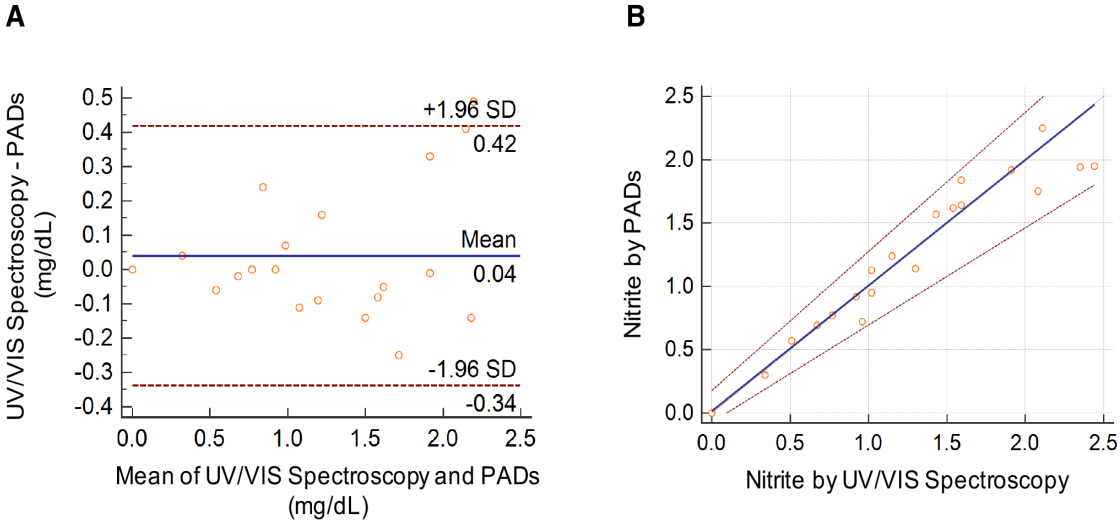



Figure S4. Comparison of nitrite levels obtained for the proposed-PADs and spectrophotometric assay. The results are shown as (A) a Bland-Altman plot and (B) a Passing-Bablok regression analysis.

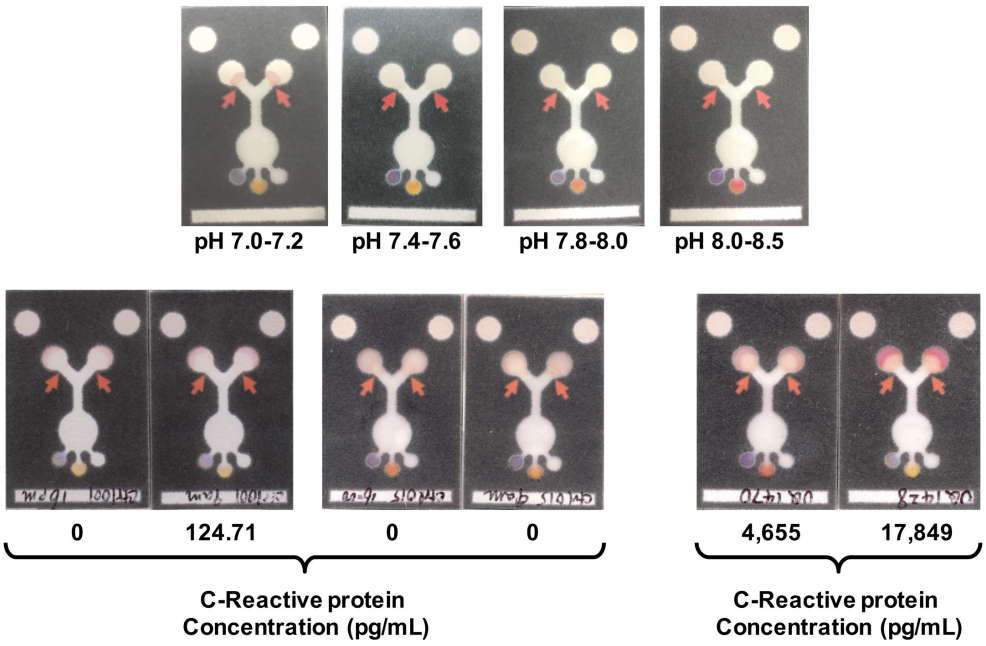



Figure S5. Real sample analysis (A) pH testing on PADs (B) Saliva contain low concentration of C-reactive protein (CRP) (C) Saliva contain high concentration of C-reactive protein (CRP)