بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

IN THE NAME OF ALLAH

Novel Anthocyanin-Based Colorimetric Assay for the Rapid, Sensitive, and Quantitative Detection of Helicobacter pylori

Advisor: DR. Poursina
Presented By: Amirhossein Tashakor

No.	Title	Subject Category	Publisher/ Holder	IF	IF Quartile	CiteScore	CiteScore Quartile	H-Index	Indexed in	Details
1	Analytical Chemistry ISSN/ISBN: 0003-2700, 1520-6882	Analytical Che... Chemistry	ACS	7.400	Q1	12.30	Q1	356	ISI, Scopus, PubMed	

pubs.acs.org/ac

Article

Novel Anthocyanin-Based Colorimetric Assay for the Rapid, Sensitive, and Quantitative Detection of *Helicobacter pylori*

Cagla Celik,[▽] Gulten Can Sezgin, Umut Gorkem Kocabas, Sebnem Gursoy, Nilay Ildiz,* Weihong Tan,* and Ismail Ocsoy*

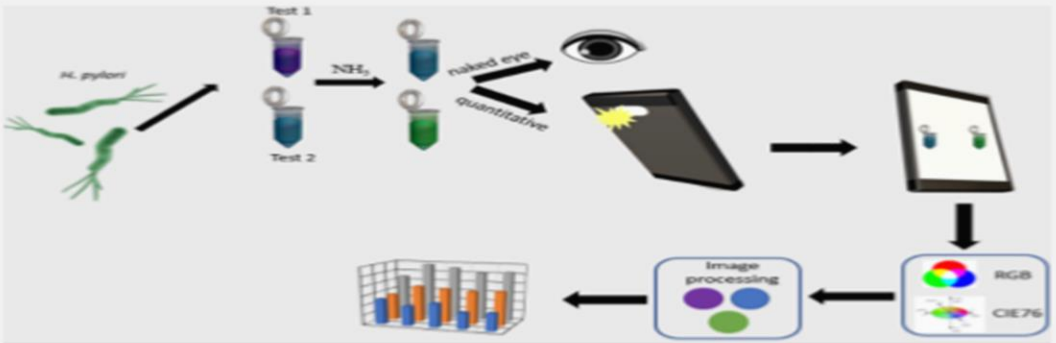
Cite This: *Anal. Chem.* 2021, 93, 6246−6253

Read Online

DOI: 10.1021/acs.analchem.1c00663

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Several different diagnostic tests have been reported for rapid, sensitive, and economical detection of bacterial pathogens, but most lack widespread and practical use in the clinic. In this study, we used anthocyanins from red cabbage (*Brassica oleracea*) as a natural pH indicator and, for the first time, incorporated this agent into a simple, rapid, and economical colorimetric strategy for the detection of *Helicobacter pylori* (*H. pylori*) (RCE@test). We prepared two sets of RCE@test solutions (test 1 is purple, and test 2 is blue) in different forms, including liquid, adsorbed filter paper, and agar, and investigated the performance of each RCE@test as a function of the test volume, *H. pylori* concentration, and reaction time. To elucidate the effect of the pathophysiological environment on these RCE@tests, *H. pylori* in an artificial gastric fluid was also detected. The 10 and 1 CFU/mL *H. pylori* suspensions were detected in 15 min and 3 h, respectively, and the limit of detection was determined down to 1 CFU/mL. We experimentally demonstrated the advantages of the RCE@test for detection of *H. pylori* by comparing it to a commercially available rapid urease test, the "CLO test (*Campylobacter*-like organism test)". In addition to colorimetric detection by the naked eyes, RGB (Red Green Blue) and Delta-E analysis in image-processing software was run to quantitatively monitor changes of color in the RCE@test using a smartphone application. Finally, we propose that this test provides simple, effective, rapid, and inexpensive detection and that it can be easily implemented for clinical use.



Outlines

1. Introduction

2. Methods

2.1. Red Cabbage (Brassica oleracea) Extract Preparation.

2.2. Bacterial Strains and Culture Conditions.

2.3. Preparation of Anthocyanin-Based Colorimetric Tests.

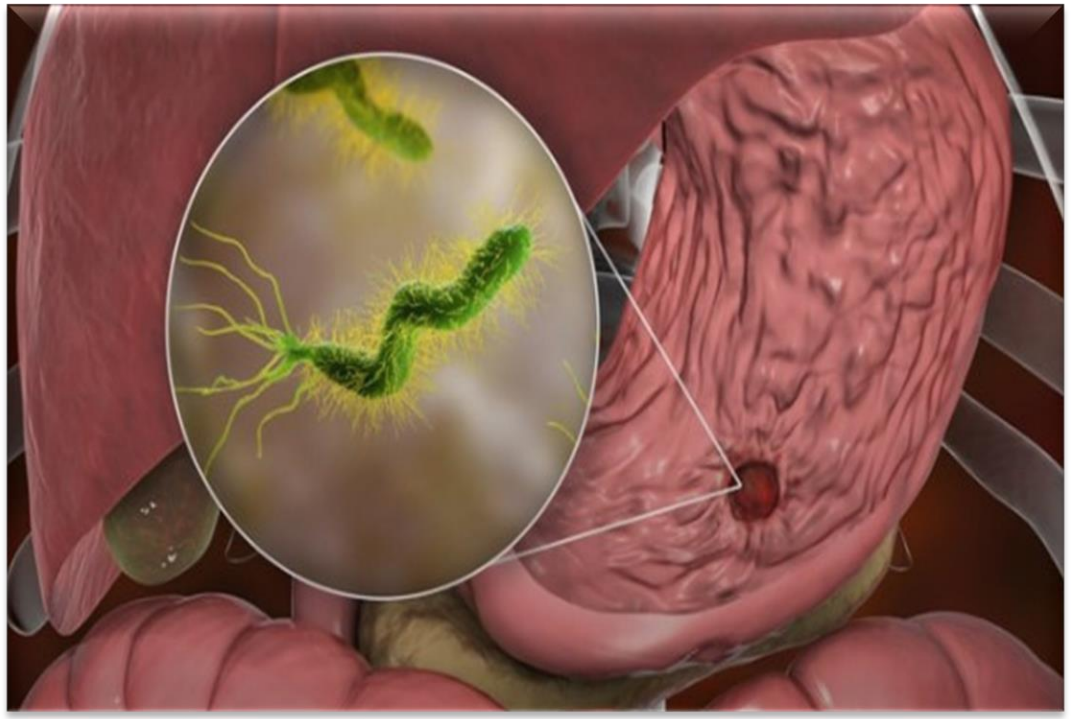
2.4. Digital Image Processing.

2.5. Development of a Smartphone Application.

3. Results

4. Discussion

5. Conclusions



Introduction

Introduction

Infections cause increased worldwide mortality each year.

Prolonged and frequent use of antibiotics raises three main problems:

Helicobacter pylori infecting between 40 and 90% of the population worldwide

- adversely harming patients' health
- developing new resistance
- negatively affecting the economy

Introduction

H. pylori colonizes in the human stomach

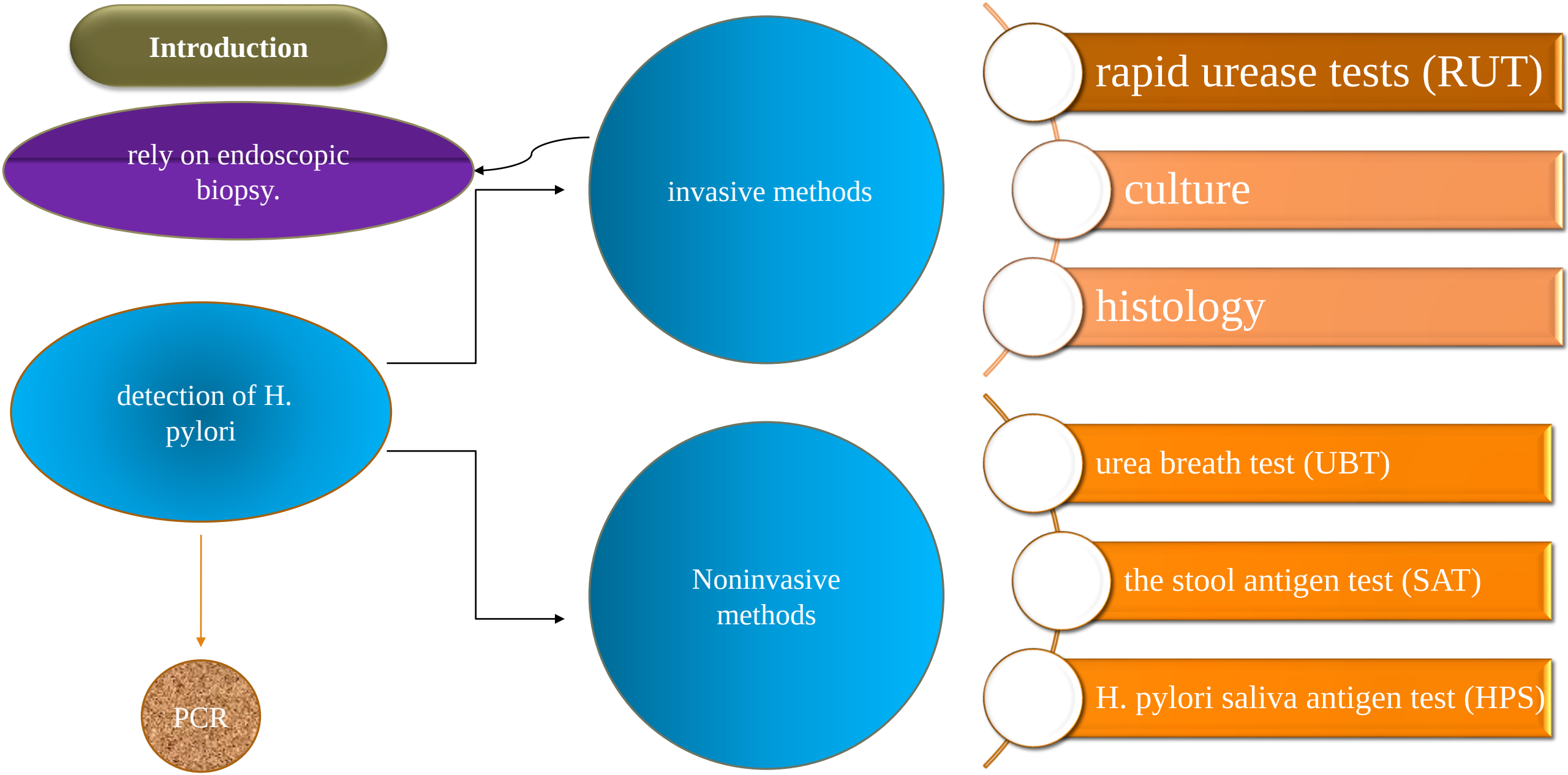
- peptic duodenal
- gastric ulcers
- gastric cancer

↓

one million deaths each year.

↓

rapid, accurate, and sensitive detection of H. pylori is quite important



Introduction

rely on endoscopic biopsy.

detection of H. pylori

PCR

invasive methods

Noninvasive methods

- rapid urease tests (RUT)
- culture
- histology

- urea breath test (UBT)
- the stool antigen test (SAT)
- H. pylori saliva antigen test (HPS)

Introduction

Recently, the design of image-processing software containing various unique algorithms has been reported for quantitative analysis of colorimetric response with analytic devices.

CIE → defines chromaticity with two coordinates

RGB (Red Green Blue)

ΔE

Delta-E

Introduction

In this study

The first time REPORT, a natural indicator incorporated colorimetric urease test detection of H. pylori.

- rapid
- sensitive
- economical

METHODS

Methods

Red Cabbage Extract Preparation

Red cabbage leaves were cut into small pieces.

100 grams of the material was placed in a 500 mL beaker containing 100 mL of distilled water.

The mixture was exposed to the extraction process by boiling for 30 min.

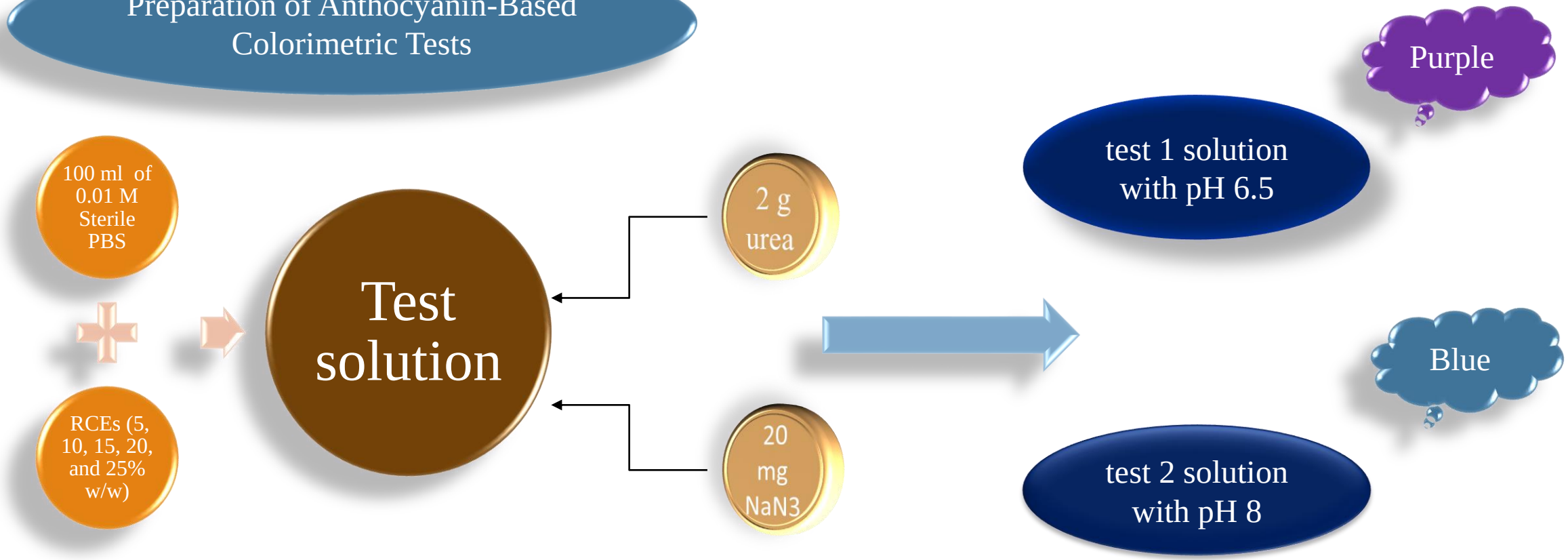
Methods

Bacterial Strains and Culture Conditions

The H. pylori strain was grown at 37 °C under microaerophilic conditions (jar with a gas-generating kit) in tryptic soy agar and Columbia agar base containing 5% sterile sheep blood for about 3 days. The H. pylori strain was suspended in 3 mL of saline at different turbidities.

Methods

Preparation of Anthocyanin-Based Colorimetric Tests



Methods

Digital Image Processing

Capture test images via a smartphone camera

Calculate RGB values in ImageJ software

- Red
- Green
- Blue

measure Delta-E (ΔE)

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

Methods

Development of a Smartphone Application

develop “INC Test” APP

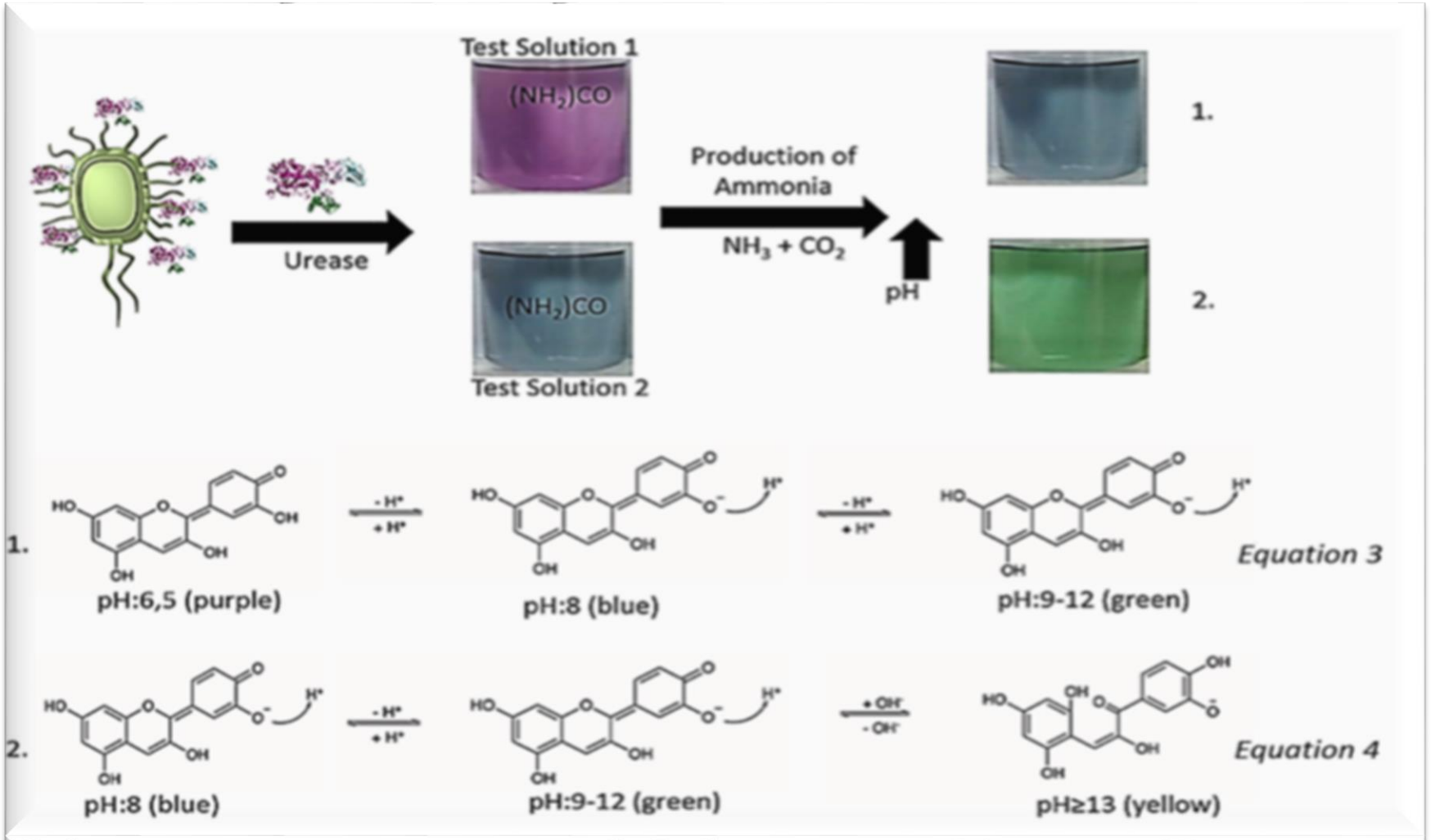
calculate RGB values

$$ED^2 = (R_2 - R_1)^2 + (G_2 - G_1)^2 + (B_2 - B_1)^2$$

positive test: values of 25 and above

RESULTS

Results



Scheme 1. Preparation of Colorimetric Tests at Two Different pH Values (Test 1 at pH 6.5 and Test 2 at pH 8) and Detection of *H. pylori* Based upon Color Changes with Potential Equations (Equations 3 and 4)

Results

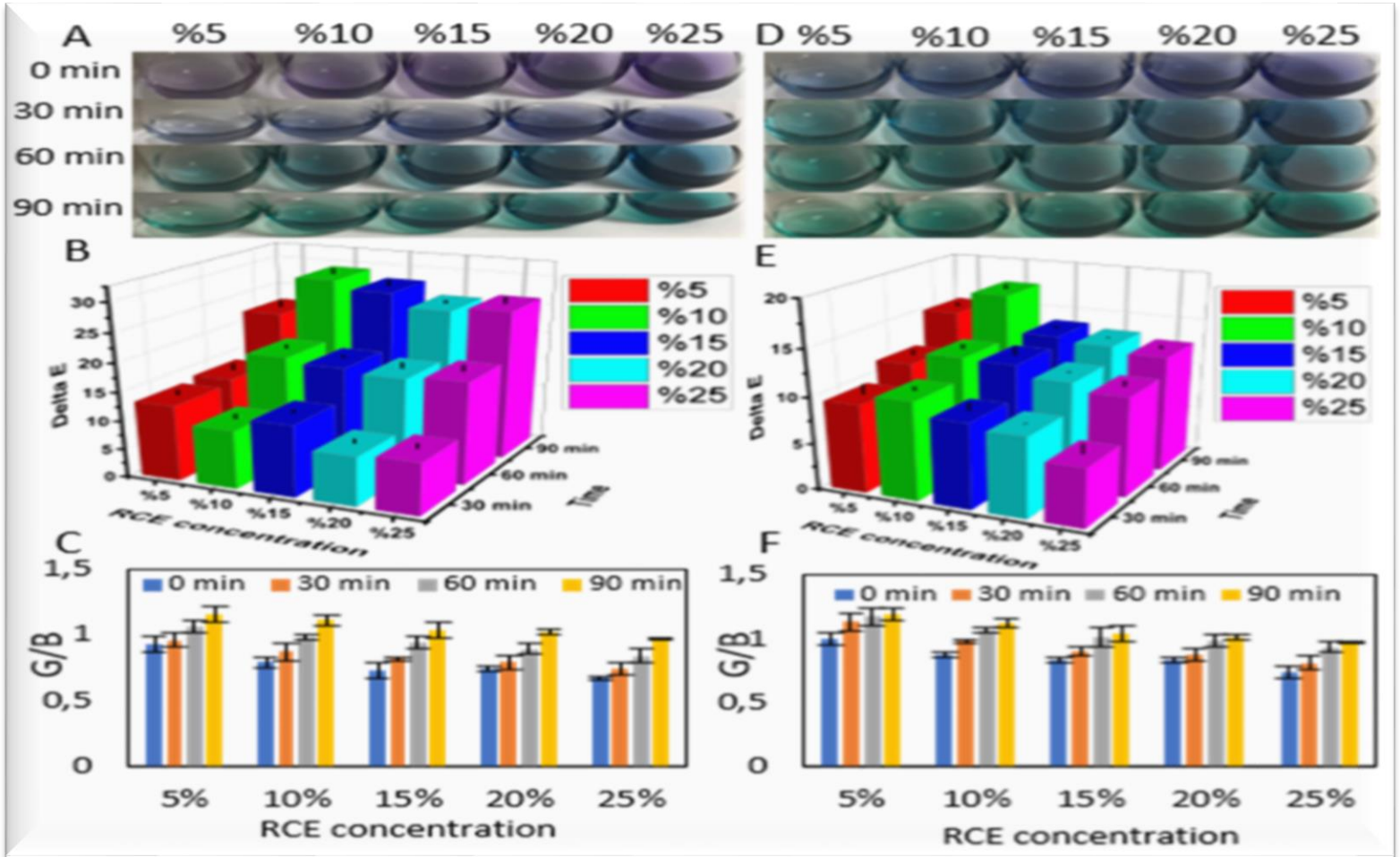


Figure 1. Test 1 and test 2 solutions prepared at (A) pH 6.5 and (D) pH 8 and incubated for 30, 60, and 90 min in the presence of *H. pylori*. ΔE analysis of (B) test 1 and (E) test 2. RGB analysis of (C) test 1 and (F) test 2. The error bars demonstrate one standard deviation (SD) obtained from three independent measurements (n = 3).

Results

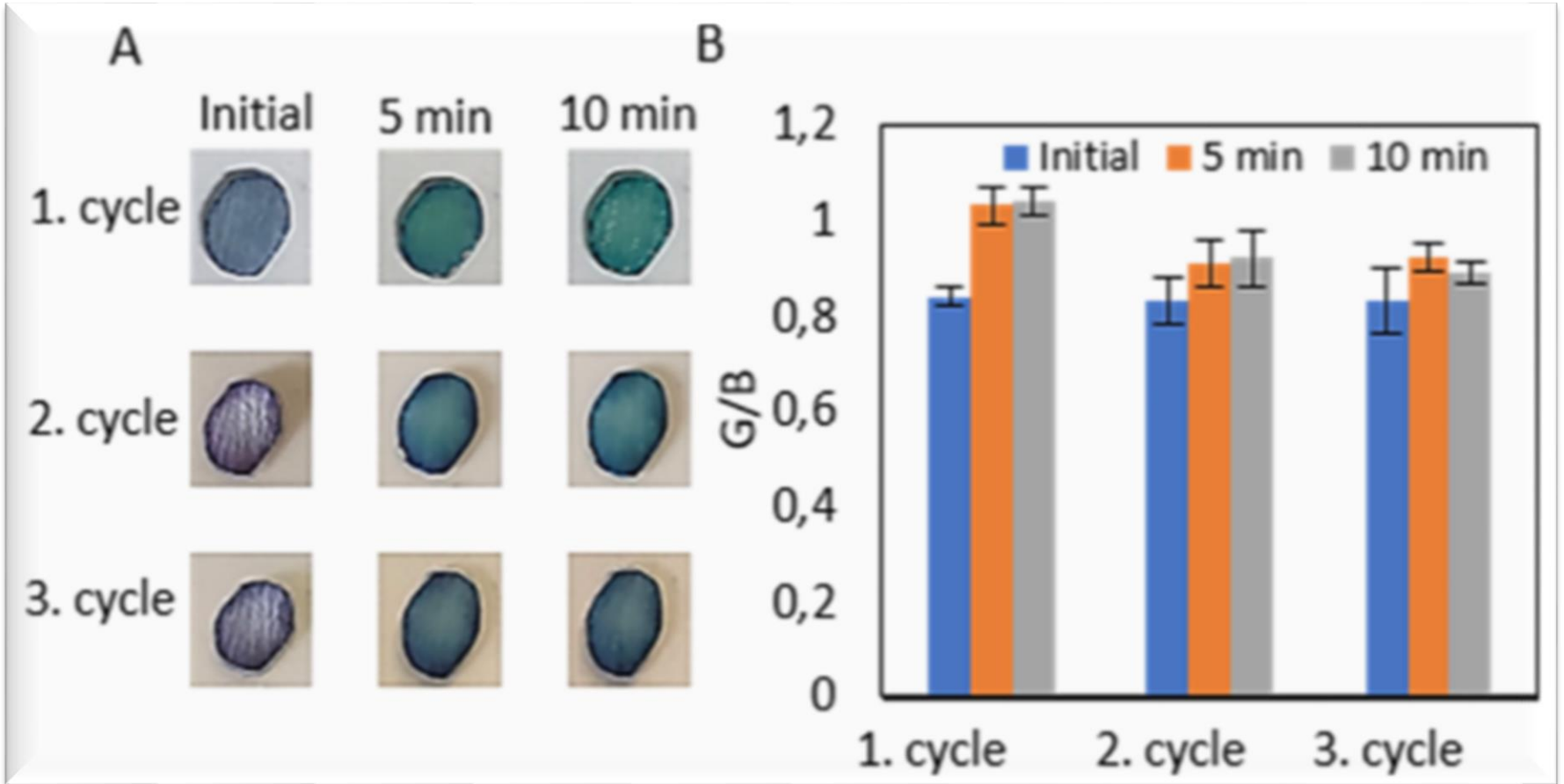


Figure 3. Performance of the test prepared on filter paper for *H. pylori* detection. Repeated use of the filter paper. (A) Naked-eye detection and (B) G/B values. The error bars demonstrate one standard deviation (SD) obtained from three independent measurements (n = 3).

Results

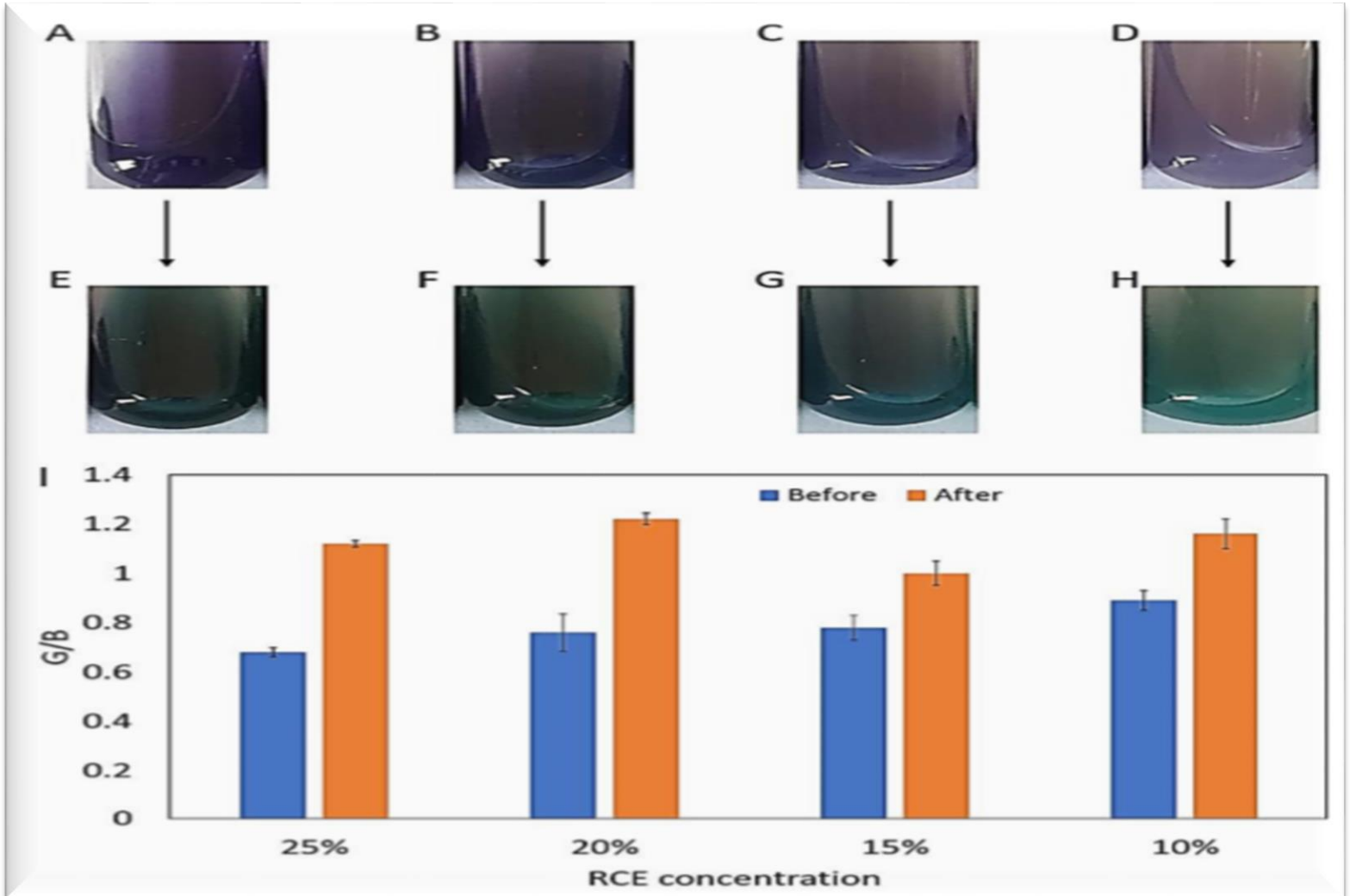


Figure S5. Detection of *H.* pylori (2x10^5 CFU/mL suspensions) using RCE-incorporated agars. A-E) 25% w/w of RCE, B-F) 20% w/w of RCE, C-G) 15% w/w of RCE, D-H) 10% w/w of RCE and I) G/B analysis.

Results

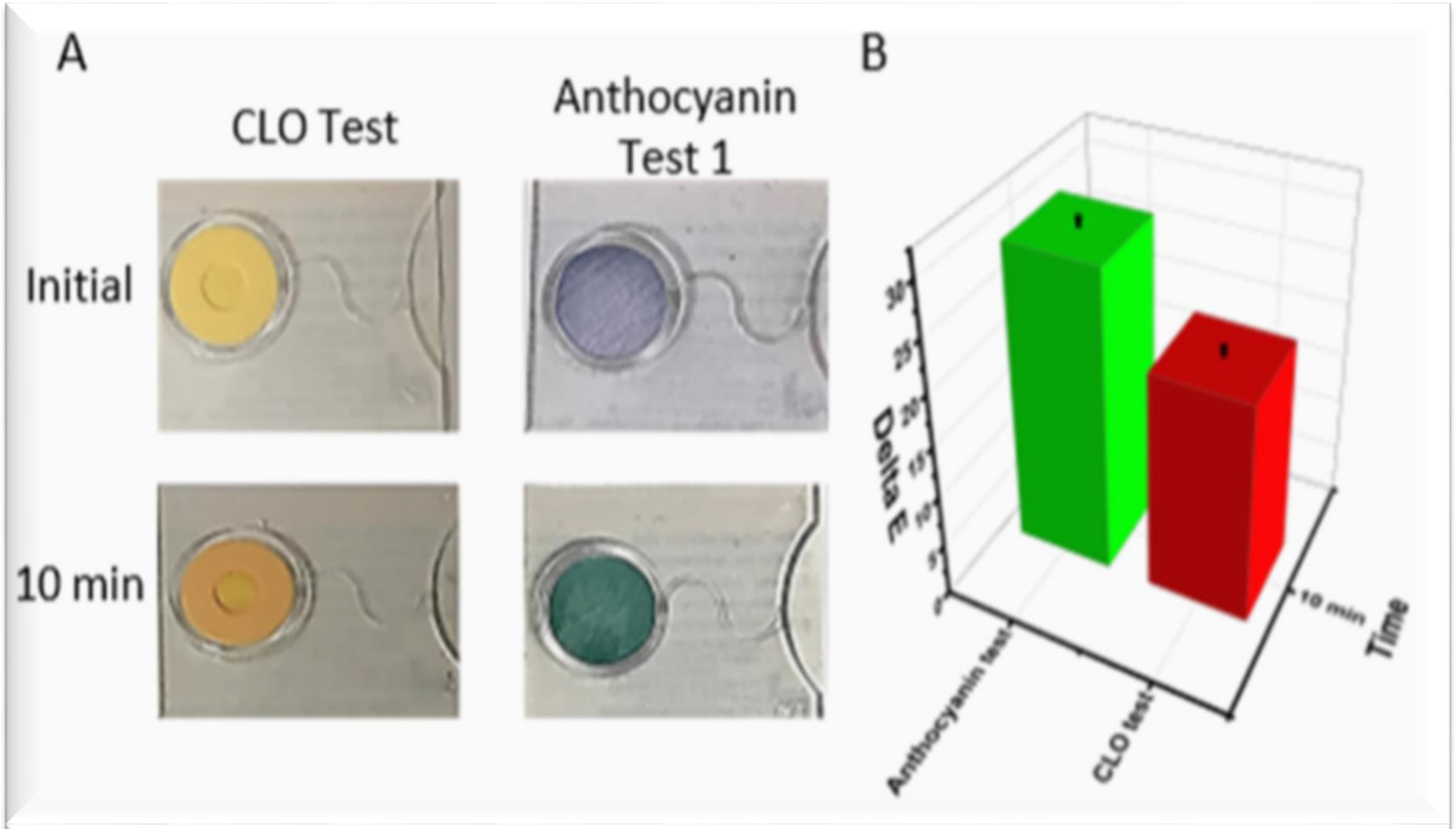


Figure 4. Comparison study for *H. pylori* detection using a CLO test and anthocyanin test 1. (A) Colorimetric response. (B) ΔE analysis. The error bars demonstrate one standard deviation (SD) obtained from three independent measurements (n = 3).

Results

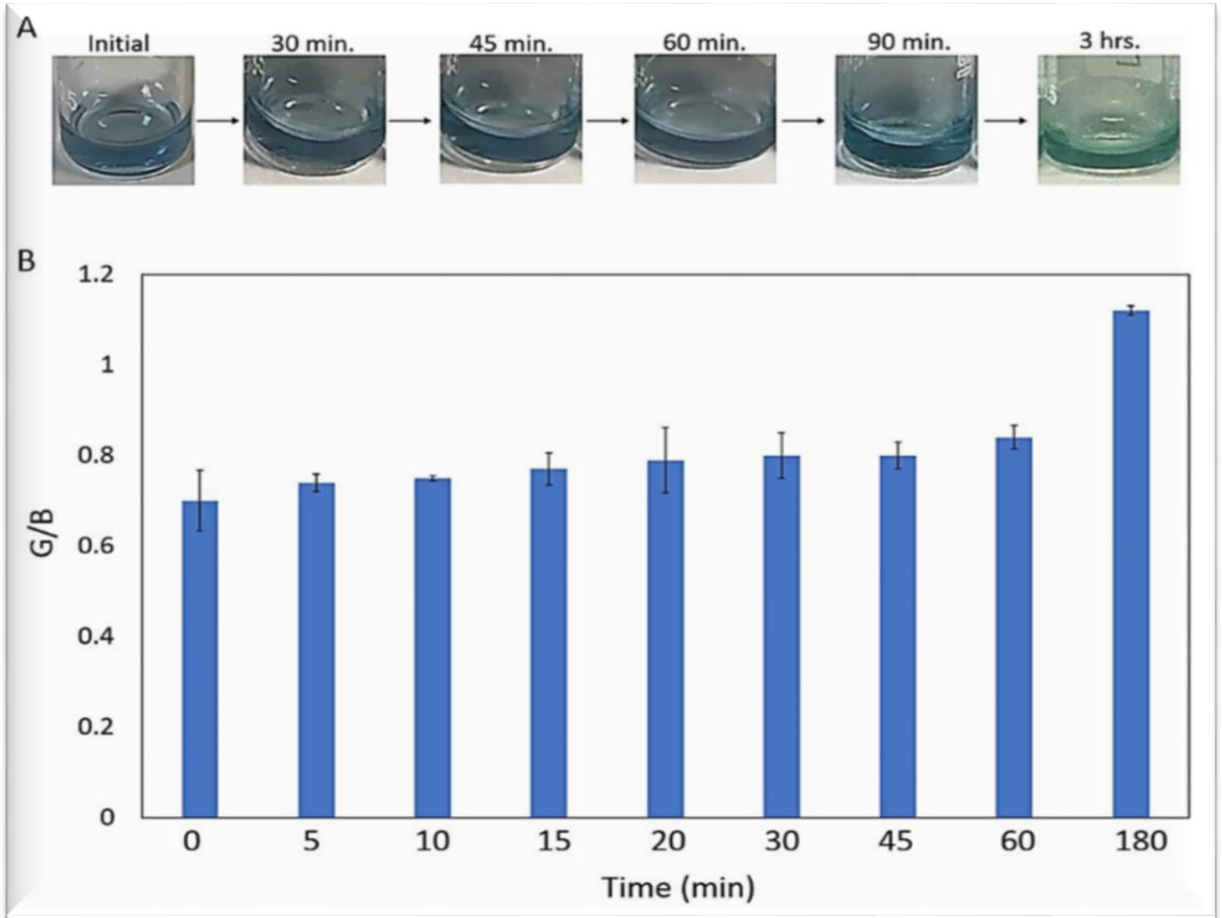


Figure S7. The sharp green color on liquid form of test 2 after 3 hrs incubation of 1 CFU/mL *H. pylori* A) colorimetric result and B) G/B analysis.

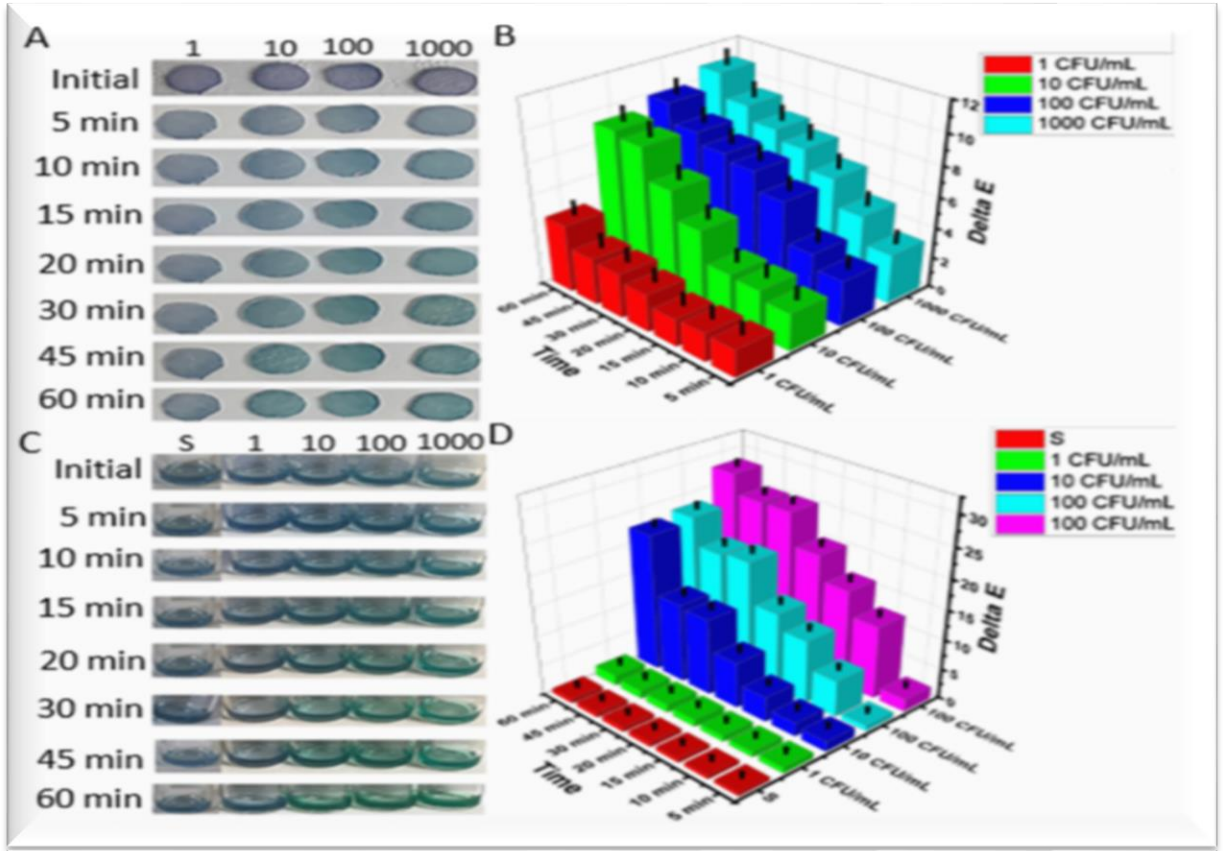


Figure 5. CFU/mL-dependent *H. pylori* detection using test 2 prepared on filter paper: (A) colorimetric response and (B) ΔE analysis and liquid-form (C) colorimetric response and (D) ΔE analysis. Error bars demonstrate one standard deviation (SD) obtained from three independent measurements ($n = 3$).

Results

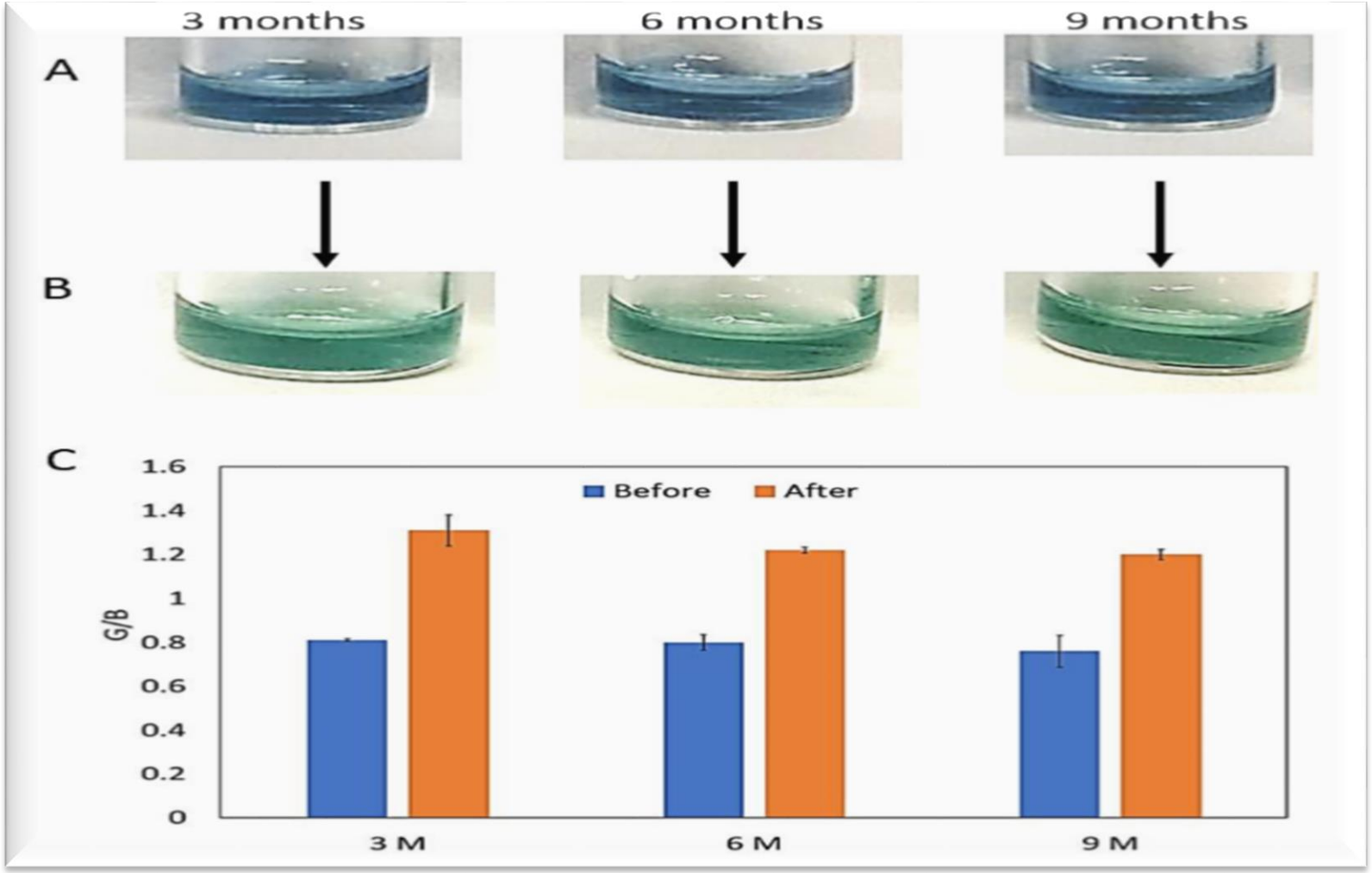


Figure S9. Excellent stability of the test 2 which stored at -20°C for 3, 6 and 9 months. A) Blue color of test 2 as a initial form B) Green color of test 2 after treatment with bacteria and C) G/B analysis.

Discussion

Discussion

Anthocyanin-rich red cabbage extract (RCE)

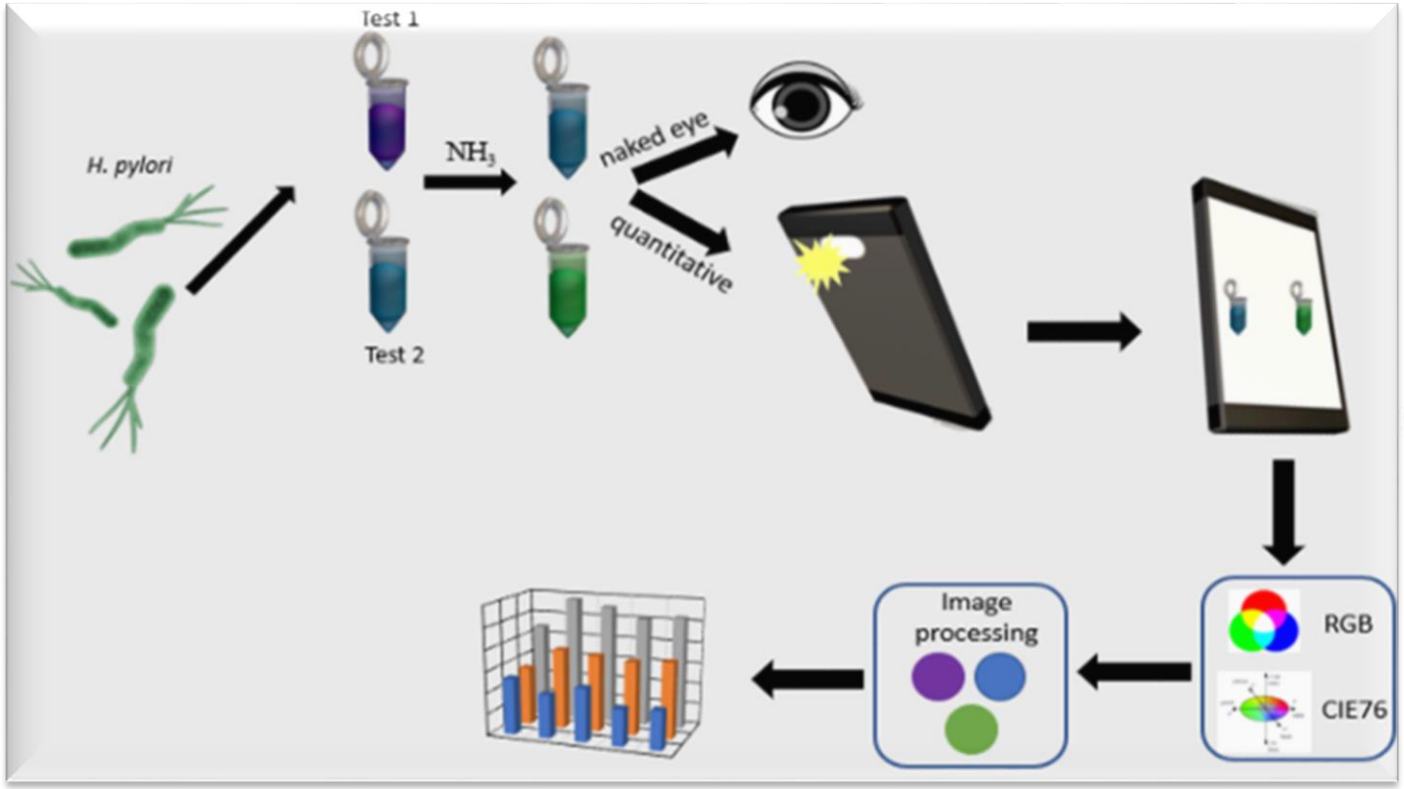
Natural indicator	FDA-approved	Very low cost	easy preparation	biocompatibility	wide availability	high stability	wide color spectrum

Discussion

Commercial Tests	Technique	Indicator	LOD (CFU/ ml)	Detection time	Reusability	Biocompatibility
Ploritek	Urea-impregnated pad	Phenol red	Not reported	1 hour	Not reported	Phenol red toxicity
CLO test	Agar gel test	Phenol red	10^4	≥4-24 hour	Only for negative samples	Phenol red toxicity
Hp One	Liquid test	Bromo-thymol blue	Not reported	1 hour	Not reported	Bromothy-mol toxicity
Pronto Dry	Dry filter-paper test	Phenol red	Not reported	1 hour	Only for negative samples	Phenol red toxicity
Hp Fast	Agar gel test	Bromo-thymol blue	Not reported	≥4-24 hour	Not reported	Bromothy-mol toxicity
Anthocyanin test	Liquid test	Anthocyanin	1	<1 hour	Both of negative and positive samples	Biocompa-tible

Figure S10. Comparison table with other commercial urease tests.

Conclusions



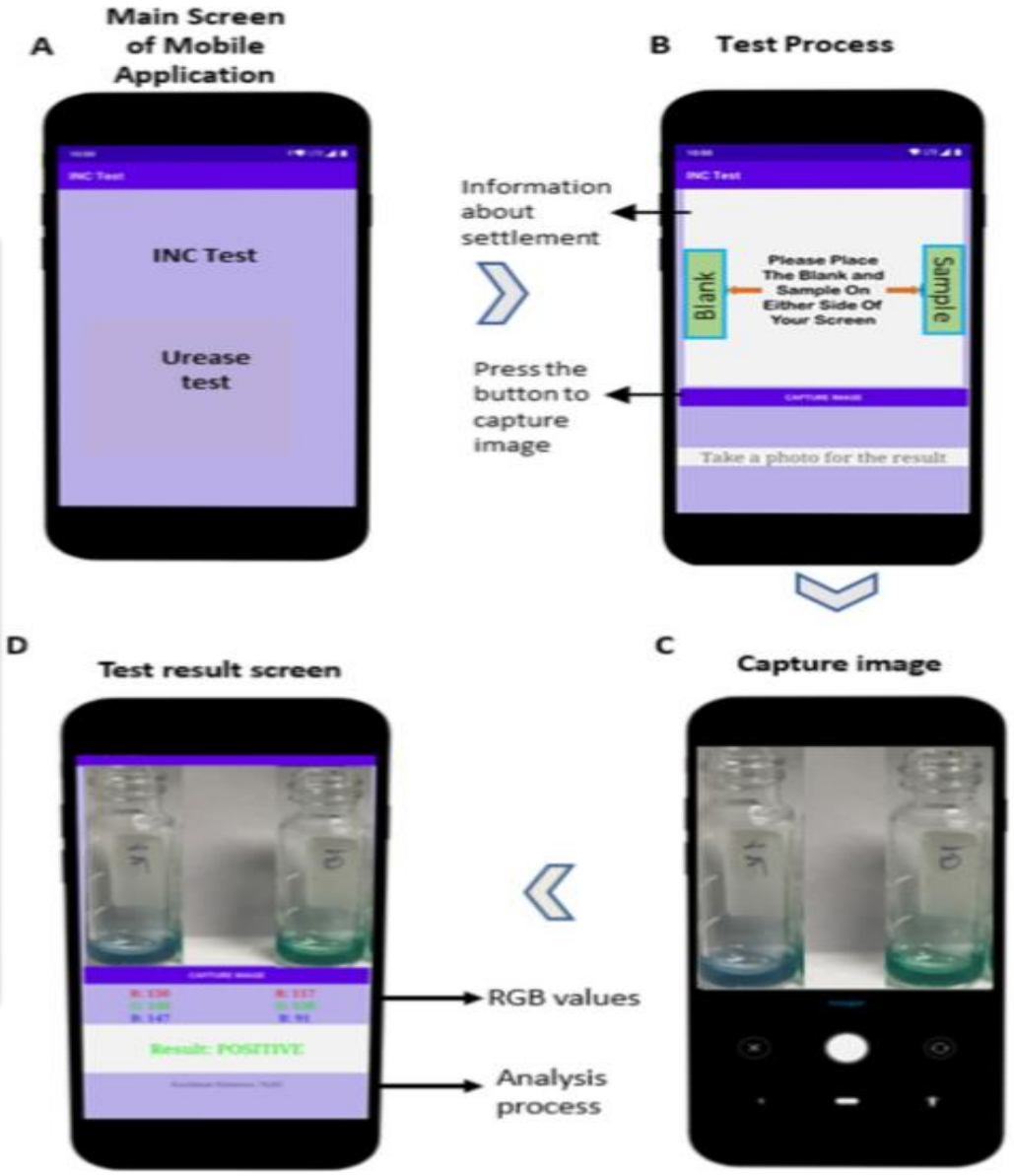


Figure 6. Smartphone-assisted *H. pylori* detection test. Each panel indicates smartphone interfaces: (A) main screen, (B) test process, (C) image capture, and (D) test result screens.

THANK
YOU