روش های تشخیص تهاجمی و غیرتهاجمی هلیکوباکتر پیلوری

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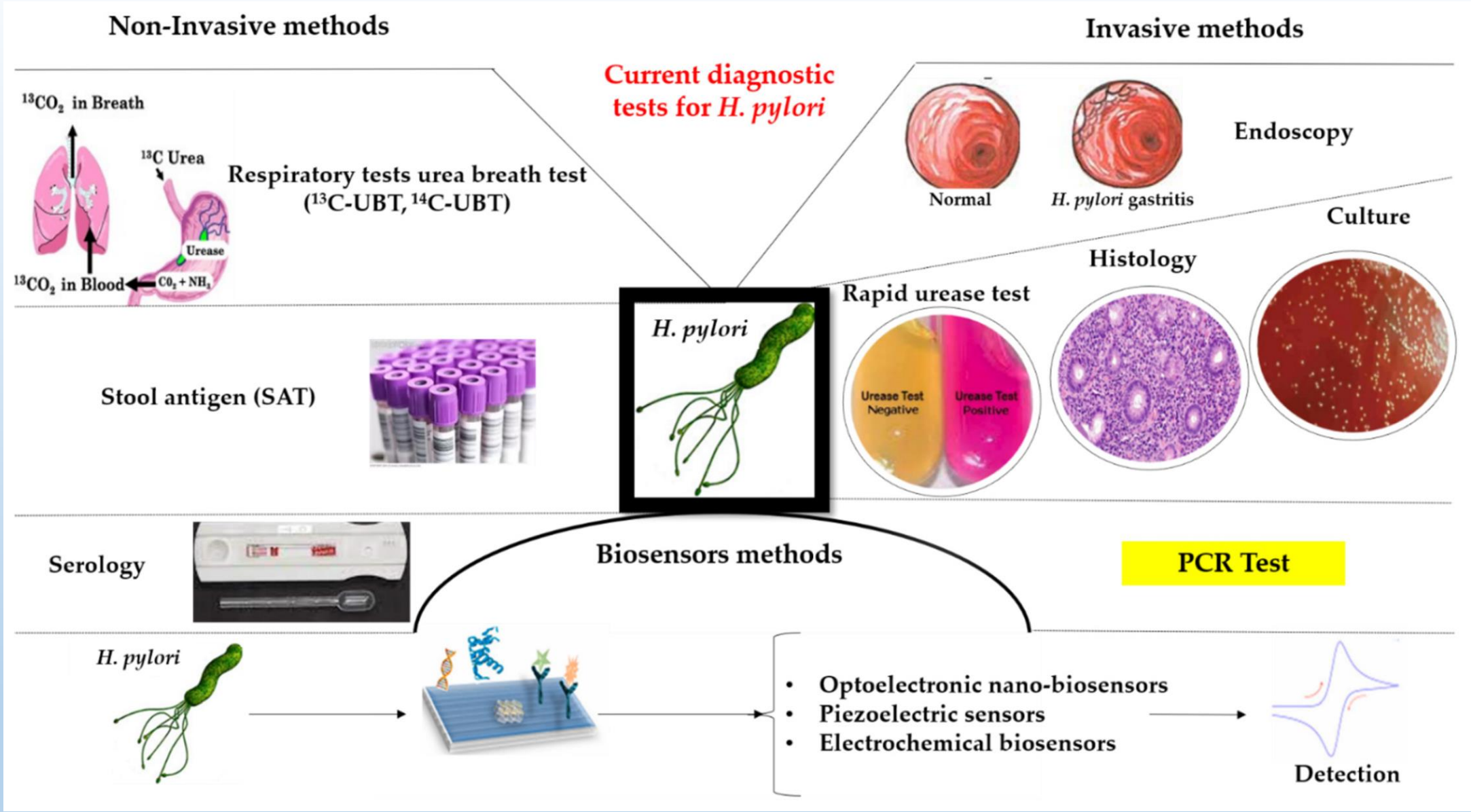
Background

- *Helicobacter pylori*
- The recommendation for young patients with dyspepsia is the ‘test-and-treat’ strategy with non-invasive tests.
- The use of one or another test depends on the accessibility of those tests, equipment from laboratories, and the clinical conditions of patients.


Non-Invasive methods
Invasive methods
Current diagnostic tests for H. pylori
13CO2 in Breath
13C Urea
Urease
13CO2 in Blood
CO2 + NH3
Respiratory tests urea breath test (13C-UBT, 14C-UBT)
Endoscopy
Normal
H. pylori gastritis
Culture
Histology
Rapid urease test
Urease Test Negative
Urease Test Positive
Stool antigen (SAT)
H. pylori
Serology
Biosensors methods
PCR Test
H. pylori
Optoelectronic nano-biosensors
Piezoelectric sensors
Electrochemical biosensors
Detection

H. pylori diagnostic tests	
Invasive	**Non-invasive**
Gastroscopy • standard videoendoscopy • high magnifying endoscopy • chromoendoscopy	**Serology** • near patient tests (HelicoTest) • ELISA
Rapid urease test (CLO test)	**UBT test C13, C14**
Histology (Giemsa staining)	**HP stool antigen test (HPSA)** • polyclonal antibody–based ELISA • monoclonal antibody –based ELISA
Microbiology Culture	**Gastropanel** • *H. pylori* antibodies • Pepsinogen I, II • Gastrin 17

Criteria for selection of Diagnostic tests

- Simple,
- Fast,
- Cost-effective,
- Portable,
- Miniaturized,
- Small volume of samples required,
- Highly sensitive, and selective.

Urea Breath Tests (UBT)

- UBT measures the difference in the proportion between $^{13}C/^{14}C$ before and after swallowing urea that is radioactively labeled in the exhaled air using mass spectrometry.
- Is a Respiratory test
- H. pylori secretes urease, which will convert urea to ammonia and neutralize the acidic pH so that it can penetrate the onset of mucus and attach to the gastric wall cells.
- Antibiotic treatment should be stopped 30 days before the test and proton pump inhibitors (PPI) should be stopped 15 days prior to taking the test.

UBT

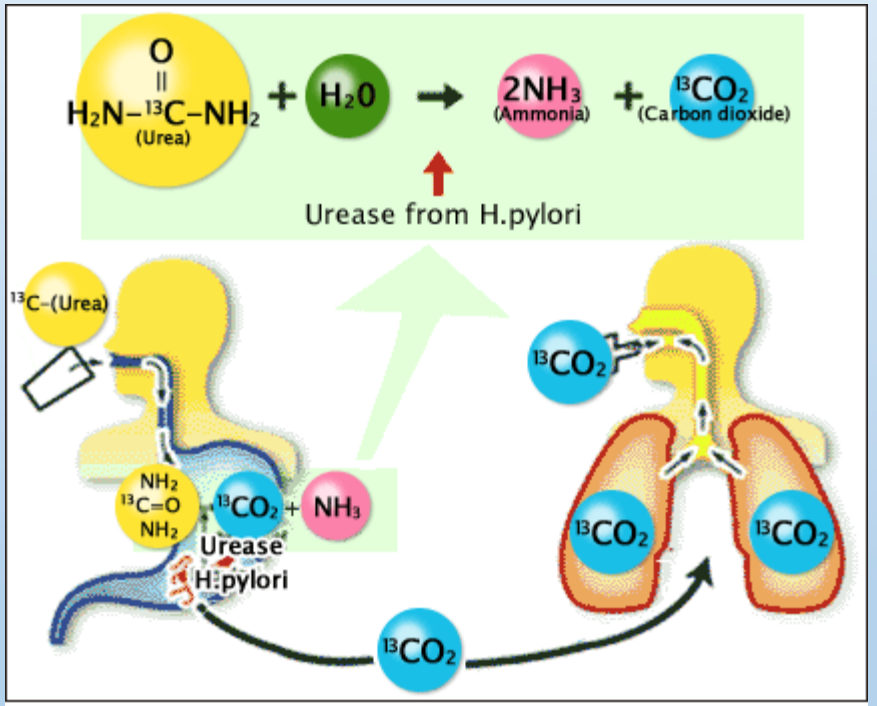
- Four samples must be collected from the patient, two before 13C-labeled urea and two after.
- Before performing the test, the patient should maintain digestive rest for at least six hours, preferably overnight.
- First, two respiratory samples are collected from the patient using the tubes or bags. The patient then receives a “test mass” followed by the administration of the 13C-labeled urea solution mixed with water. After half an hour, two more breath samples are taken. Children that are from 3 to 11 years old, should take a “test table” that contains 100 mL of orange juice.

UBT-Continued

- The active substance containing **13C-labeled urea** is labeled with carbon-13 isotope (13C), a rare form of carbon atom, instead of carbon-12 (12C), the most common form.
- The ureases contained by *H. pylori* promote the transformation of urea into carbon dioxide.
- Patients who received 13C-labeled urea in the test, and are infected, exhale carbon dioxide that contains 13C. This form of **labeled carbon dioxide** can be measured in laboratories using either isotope ratio mass spectrometry (IRMS), non-dispersive isotope-selective infrared spectroscopy (NDIRS), or laser-assisted ratio analyzer (LARA).
- The test is considered positive if there is marked carbon dioxide in the respiratory sample taken after 30 min. The absence of it results is a negative result. This method is useful both for adults and **children who are 3–11 years old.**
- 13C-UBT sensitivity and specificity for detection of H. pylori infection were 76.2% and 69.2%.

	14C-UBT	13C-UBT
Test performed at	Nuclear medicine department	No specific location required
Analysis	Specialized nuclear medicine department and β-scintillation counters	Mass spectrometry analysis (in a hospital or mailed to the manufacturer)
Radioactive hazard	Yes	No
Patient selection	Not suitable for children or pregnant women	Safer for children or pregnant women

UBT: Urea breath test; 14C: 14 carbon; 13C: 13 carbon.



Principle of urea breath test

SUMMARY TABLE: ACCURACY OF *HELICOBACTER PYLORI* TESTING AT A PREVALENCE OF 53.7% AND SPECIFICITY OF 90%

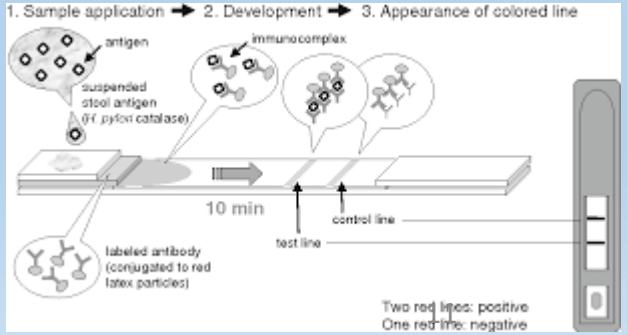
Test	Sensitivity percentage (95% CI)	Diagnostic odds ratio (95% CI)	False-negative rate per 1,000 patients (95% CI)	Positive predictive value percentage
Urea breath test-^{13}C	94 (89 to 97)	153 (73.7 to 316)	30 (15 to 58)	92
Urea breath test-^{14}C	92 (89 to 94)	105 (74 to 150)	42 (30 to 58)	92
Serology	84 (74 to 91)	47.4 (25.5 to 88.1)	86 (50 to 140)	91
Stool antigen	83 (73 to 90)	45.1 (24.2 to 84.1)	89 (52 to 146)	91

Ref: **Non-invasive diagnostic tests for Helicobacter pylori infection.** 2018 Mar 15;3(3):CD012080.

The recommendation for *H. pylori eradication* is the use of UBT. The test should be taken at least 4 weeks after the patient has finished taking the eradication therapy

Stool Antigen Test (SAT)

- SAT can detect *H. pylori* antigens in human stool.
- Has an accuracy of over 90%.
- Is a quick test, useful both for diagnosis and for confirming the presence of bacteria after treatment.
- The method does not require the prior preparation of the patient, but a 2-week restriction of proton pump inhibitor (PPI) use, and a 4-week restriction of antibiotics and bismuth compounds, before testing, is recommended.



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Serology

- It is currently based on the quantitation of immunoglobulin G antibodies against H. pylori by the means of an enzyme-linked immunosorbent assay (ELISA).
- Their sensitivity is quoted at 80–95% (some other studies, 55-100%) and specificity at 80–95% (some other studies, 59.6% to 97.9%).
- IgG antibodies against *H. pylori* appear about three weeks after the onset of infection and remain high throughout the infection, returning to normal in about 1 year.
- Up to 50% of the asymptomatic adult population has a positive serology for H. pylori infection.
- The disadvantage of serology is that it cannot distinguish recent infection from past infection because the antibodies can remain detectable for several years after infection and is not useful in evaluating the rate of post-therapy eradication.

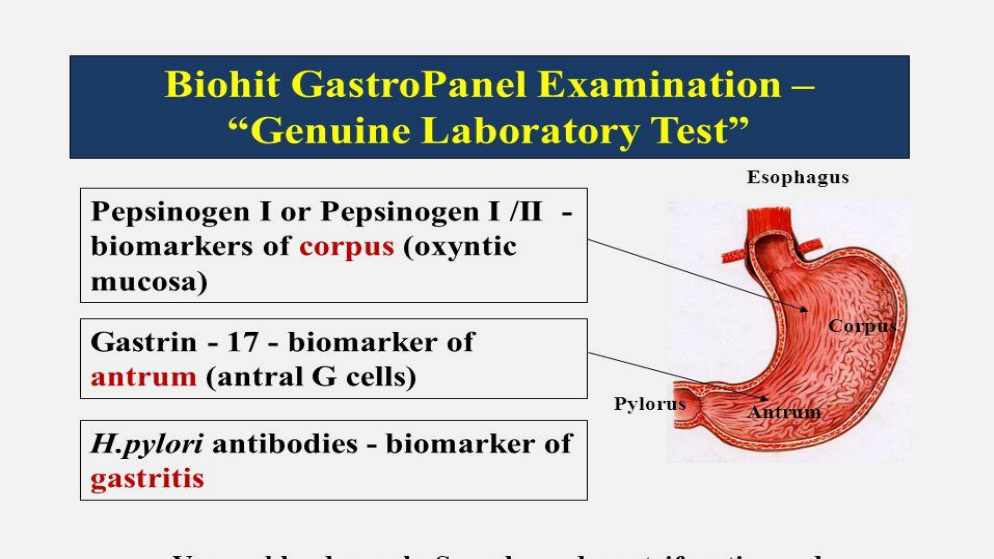
Serology-continued

- The results are not false positive in case the patient undergoes proton pump inhibitor therapy or other medications.
- Accuracy depends on what type of antigen is contained by the chosen kit, and if that strain is highly prevalent in the region it is used.

GastroPanel

- The test is useful for the diagnosis of chronic atrophic gastritis measuring four biomarkers in blood: basal gastrin-17 (G17), pepsinogen I and II (PGI and PGII), and H. pylori antibodies.

- Are useful for identifying patients who have a high risk of gastric neoplasia.



Gastropanel

- **Revolutionary Point-of-Care Stomach Health Test**
- **Reveals *Helicobacter pylori* infection, atrophic gastritis and high acid output of stomach**
- **measurement from fingertip blood sample – results available in 15 minutes**
- **can be performed during a clinical appointment**
- **speeds up the referral to further examinations**
- **GastroPanel® Quick Test is the further development of the unique Biohit GastroPanel® examination. It allows fast diagnosis of and screening for *Helicobacter pylori*, atrophic gastritis with related risks as well as high acid output of stomach in symptomatic and asymptomatic patients.**

Invasive methods
Endoscopy

- Endoscopy is one of the invasive methods of diagnosis, recommended for patients with dyspepsia aged <45–50 years.
- color imaging (LCI) and blue laser imaging (BLI)
- BLI remains the best method of diagnosing metaplasia
- Endoscopy will provide biopsies that will be useful for other invasive tests, such as histological examination, rapid urea test that detects active infections, or for H. pylori cultures.
- at least six biopsies from the antrum, large and small curves, and the middle of the gastric body is needed for diagnosis.
- For suspicious lesions, ulcerations, and focal lesions, they require additional biopsies.

Histology

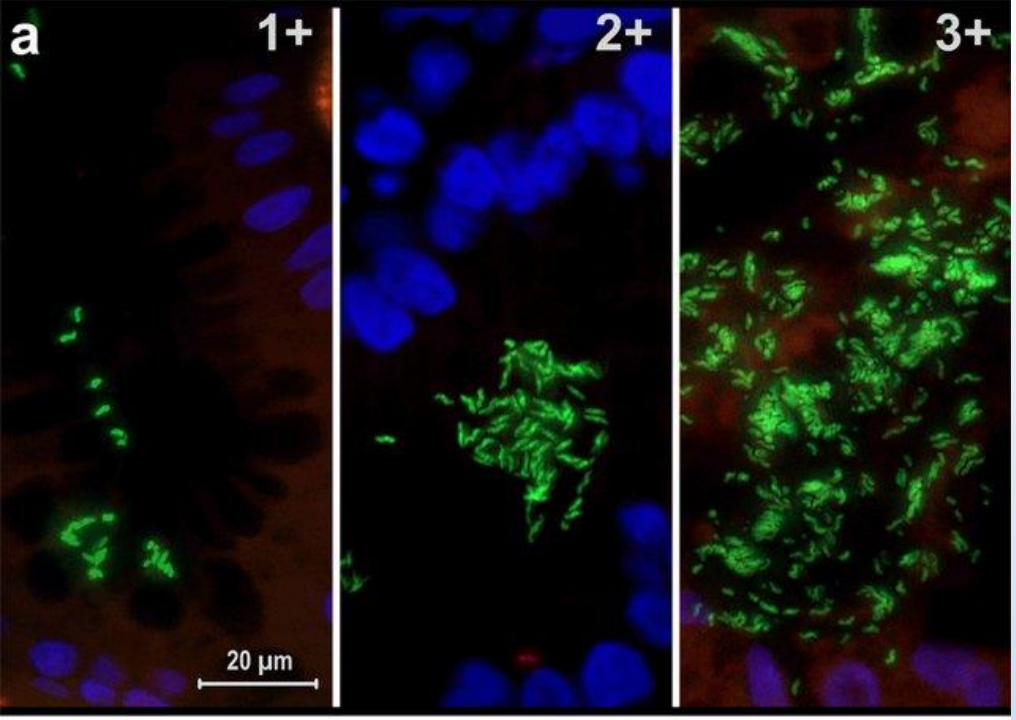
- Several factors, including the location, size, and quantity of samples, staining procedures, proton pump inhibitor (PPI), antibiotics, and the examining pathologist's experience, all influence the diagnostic accuracy of histology.
- Maastricht recommendations include stopping treatment with PPI at least two weeks before the histological examination. Biopsies involve multiple samples from the antrum and gastric body. HE staining, Giemsa, Warthine-Starry, Hp silver stain, toluidine blue, acridine orange, McMullen, Genta, Dieterle, and immunohistochemical stain are the most common stains used in practice.

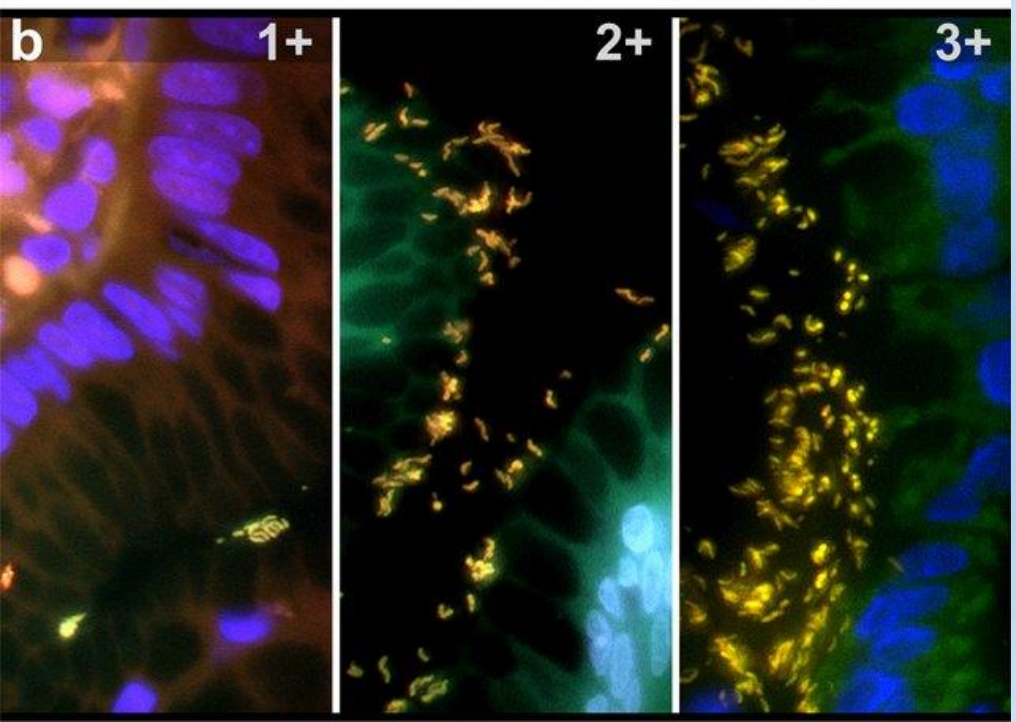
Histology (Continued)

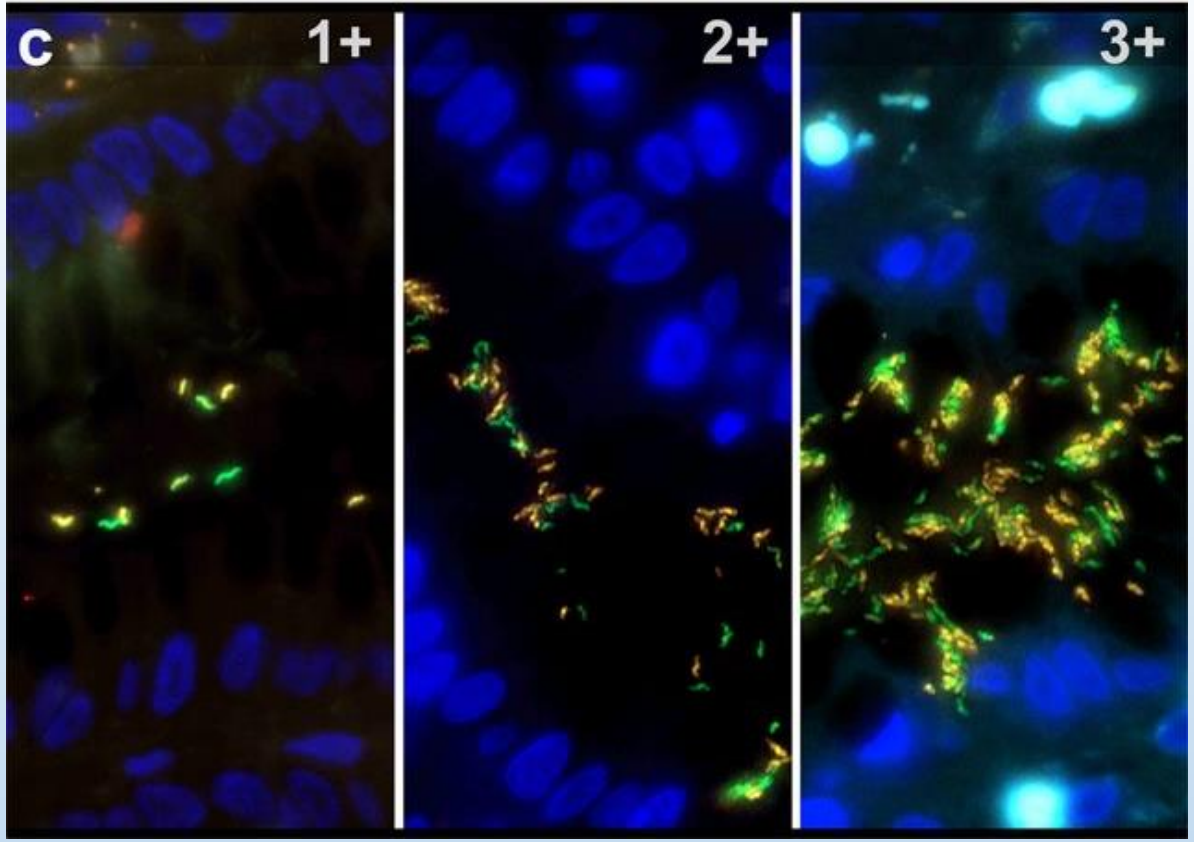
- In clinical practice, hematoxylin staining eosin HE and Giemsa are the most commonly used and least expensive.
- Immunohistochemistry is the most visible and specific staining; however, it is not always available and is more expensive.
- It has been reported that hematoxylin-eosin stain alone can detect H. pylori with a fair sensitivity between 60% and 80% of cases (FN and FP, 19%), but with low specificity (75%) compared with Giemsa stain (90%) and IHC (100%).
- Giemsa stain has a lower sensibility compared to H&E, but with higher specificity and more important issues with a lower false-positive rate.

PNA-FISH

- Fluorescent nucleic acid peptide in situ hybridization (PNA-FISH) has a specificity of 100%, identifies undetectable forms in routine staining, and is fast with good cost-effectiveness for identifying clarithromycin-resistant HP.
- The disadvantage consists of laborious preparation, a special microscope with fluorescence, and experience in reading the preparations.
- The specificity and sensitivity of peptide nucleic acid-fluorescence in situ hybridization
- PNA-FISH has 90.9 percent and 84.2 percent specificity and sensitivity, respectively, when compared to the reference method. The approach had a sensitivity of 80.0 percent and a specificity of 93.8 percent for detecting *Helicobacter pylori* clarithromycin resistance.





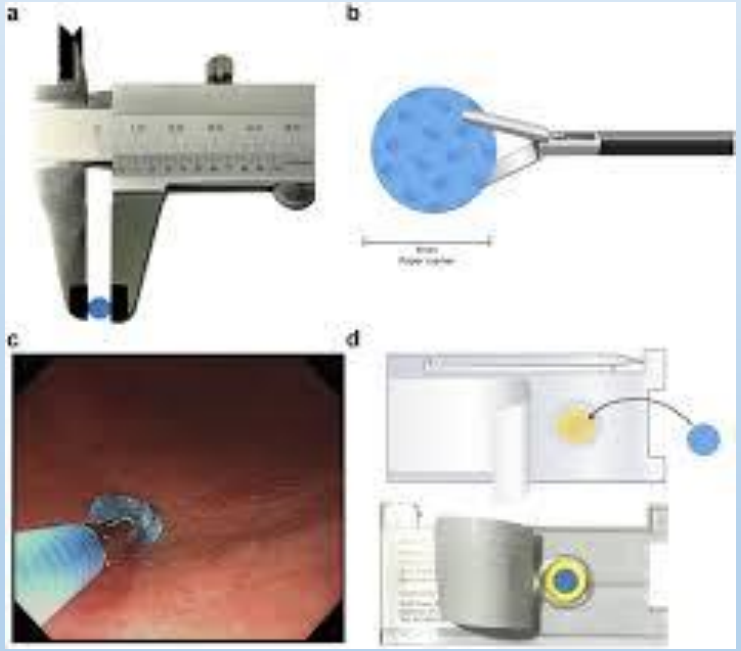


- Detection of Helicobacter pylori bacteria with fluorescence in situ hybridization (FISH) in gastric tissue samples a Clarithromycin-susceptible bacteria appear in green. b In clarithromycin-homoresistant infection, **resistant** H. pylori bacteria show yellow fluorescence. c In clarithromycin-heteroresistant H. pylori infection, a mixed population of susceptible (green) and resistant (yellow) bacteria is present. 1+/2+/3+: low/moderate/high H. pylori density. Original magnification: 1000×. For further details, see “Clarithromycin susceptibility FISH test” in the Methods.

clo-test.webp

Rapid Urease Test

- The test detects H. pylori without the need for incubation. There are a variety of commercial urease tests available, including gel, paper, and liquid-based assays with reaction times ranging from 5 min to 24 h.

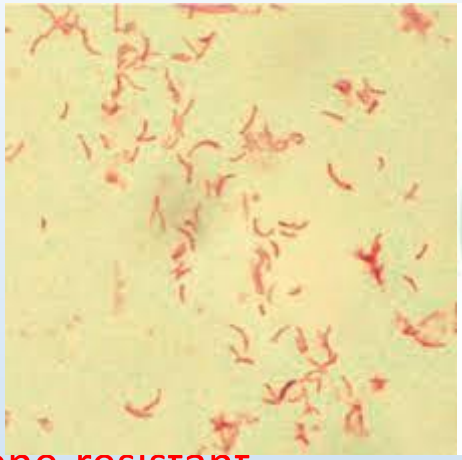


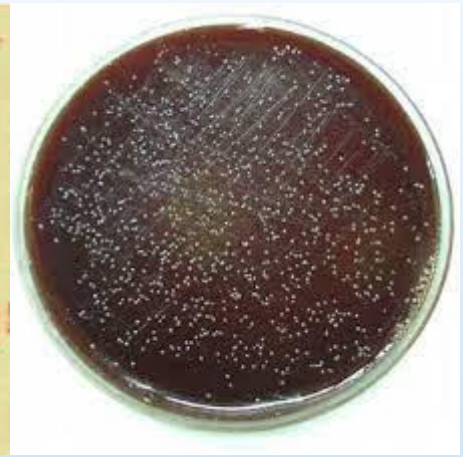
- The test started with phenol red, a color indicator that turns from yellow to pink or red when the pH rises by ammonia.

RUT (Continued)

- Clinical studies have proven the accuracy of the test to be at 90% sensitivity and 95–100% specificity
- Generally, 10^4 CFU is needed for a positive reaction.
- The presence of urease from other Helicobacter spp. can influence specificity, and false positives can occur when additional urease-producing bacteria are present: *Proteus mirabilis*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Enterobacter cloacae*, *Staphylococcus aureus.*
- Patients with achlorhydria, recent gastroduodenal hemorrhage, or usage of proton pump inhibitors (PPIs), antibiotics, H2-receptor antagonists, bismuth-containing substance, or severe atrophy and intestinal metaplasia are at risk of false-negative results.
- it is recommended that these medicines be avoided before RUT (two weeks for PPIs and 4 weeks for antibiotics).
- To maximize the sensitivity of RUTs, the current recommendation recommends obtaining at least two biopsy specimens from the stomach body and the antrum if gastroscopy is conducted

Culture





- Culture is less sensitive but a highly specific method (100%).
- It can show active infection

- Is a reference method for detecting clarithromycin and fluoroquinolone-resistant
- Need well equipped laboratories
- Need certain circumstances, beginning with collection, transport, growth, and incubation.
- Biopsy samples can be kept at 4 oC for up to 24 h in a transport medium (Portagerm pylori, Stuart's transport medium).
- Columbia blood agar, Pylori agar, Brain heart infusion, or Trypticase soy agar, supplemented with sheep or horse blood, can be used for culturing.
- Need a microaerobic atmosphere (80–90% N2, optimally closer to 10% percent CO2, 5–10% O2)
- Need examination for morphological characteristic as well as biochemical properties, such as positive urease, catalase, and oxidase reactions and MALDI-TOF-MS if the laboratory is equipped with it.
- Host factors like low bacterial load, use of PPIs, antibiotics, alcohol, and bleeding can change the culture-positive rate. Antibiotics should be avoided for at least four weeks prior to culture, and at least two biopsy specimens from the antrum and two biopsy specimens from the corpus should be obtained.

Molecular methods

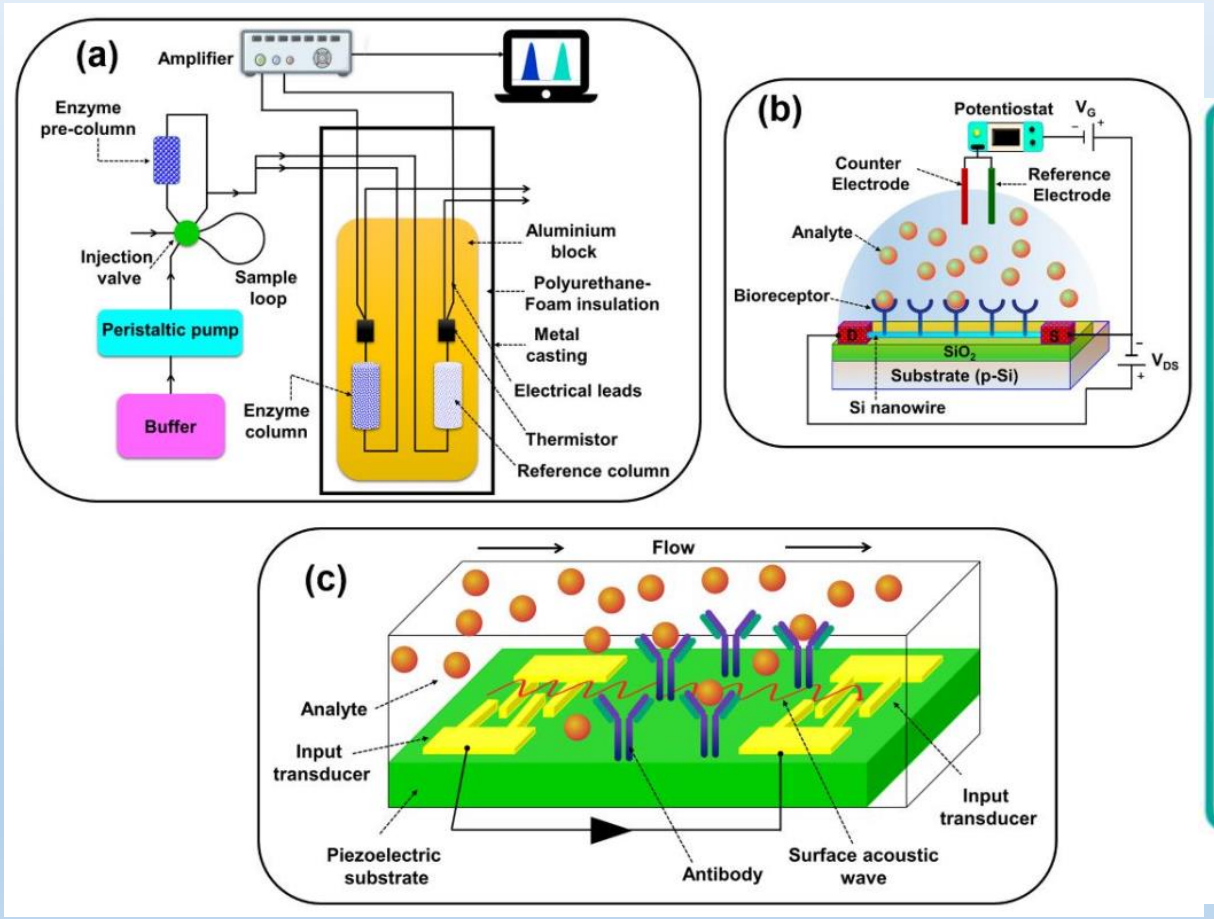
- PCR is utilized not only for detection but also for the characterization
- of pathogenic genes and antibiotic resistance mutations.
- PCR for cag-PAI and vacA alleles could predict the virulence potency.
- Clarithromycin resistance: A2143G, A2142G, and A2142C mutations
- Can be performed on urease test specimens supplied
- Has limited sensitivity detection in stool samples, with the low copy number of target DNA and the presence of PCR inhibitors in stool samples being suggested as possible causes
- Cost, local available equipment

Electrochemical methods

- Biosensors and nano(bio)sensors:
- A biosensor is designed to combine a biorecognition component (bioreceptor) with a transducer, converting the biological activity into a quantified signal.
- Two types exist: bio-catalytic (Enzyme, protein, aptamer, antibody, …) or bioaffinity-based systems

Table 2. Nano-biosensors for rapid detection of *H. pylori*.

Detection Technique	Biosensor Design	Detection Limit
Fluorescence/FRET (Fluorescence Resonance Energy Transfer)	CdTe Quantum Dots/NH_2 and Tamra labeled oligonucleotide, hybridization with *H. pylori* urease gene	4.5×10^{-9} M
	$CuInS_2$ Quantum dots/modified ssDNA/graphene oxide genosensor	0.46 pmol·L^{-1}
Fluorescence/Lateral flow immunochromatographic assay (LFIA)	Water-soluble Quantum dots-labeled urea-enzyme antibody	5 mIU/mL
Autofluorescence	Self-assembled glass-immobilized DNA-labeled AuNPs, hybridization with cDNA	5.10×10^{-10} M
Colorimetric detection	Thermophilic helicase-dependent isothermal amplification (tHDA) and AuNPs	10 CFU mL^{-1}
Aptamer-binding fluorescence methods	HPA-2 DNA aptamer with high binding abilities to *H. pylori* cells	88 CFU/mL
	HP4 Aptamer with high affinity to *H. pylori* in physiological conditions	26.48 ± 5.72 nmol/L
Fluorescence microscopy, electronic detection, wireless	Graphene printed onto water-soluble silk, functionalized with antimicrobial peptides	~100 *H. pylori* cells
Piezoelectric array	Sandwiched QCM, enzymatically amplified IgG in *H. pylori*	Not mentioned
	Piezoelectric chemical sensors functionalized with sorbent films, measuring ammonia and carbon dioxide concentrations	Not mentioned
Electrochemical	β-cyclodextrin (Au electrode)	0.15 nM
	AuNPs/Ruthenium complex (Au electrode)	25 pM
	AuNPs/Ruthenium complex (Au electrode)	12 fM
	Osmium complex (Au electrode)	6 pM
	Schiff ligand (Au electrode)	8 uM
	Ti_3C_2Tx + AuNPs (glassy carbon electrode)	1.6×10^{-16} M
	MWCNTs + Bi (carbon paste electrode)	0.06 ug/mL
	Au electrode	34 aM (target DNA) 1.3 pg (HP DNA)
	GO + AuNPs (glassy carbon electrode)	27 pM
	Au electrode	0.17 nM
	ZnO tetrapods	



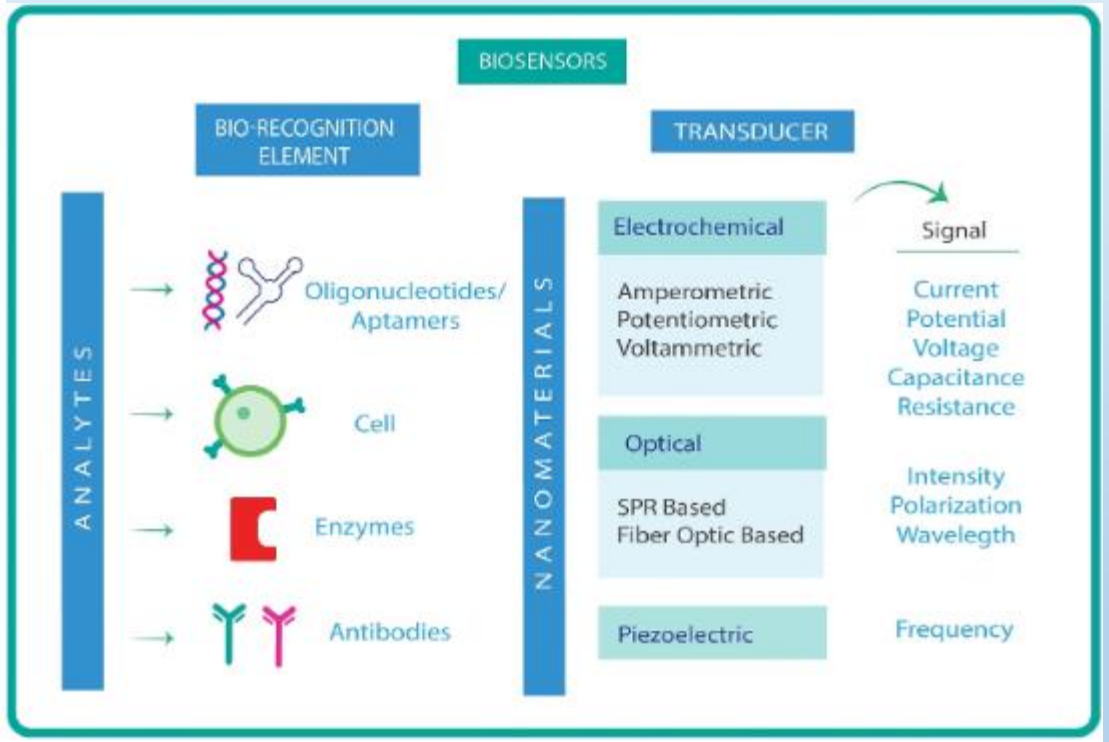


Fig. 1. Showing the different components involved in fabrication of a biosensor.

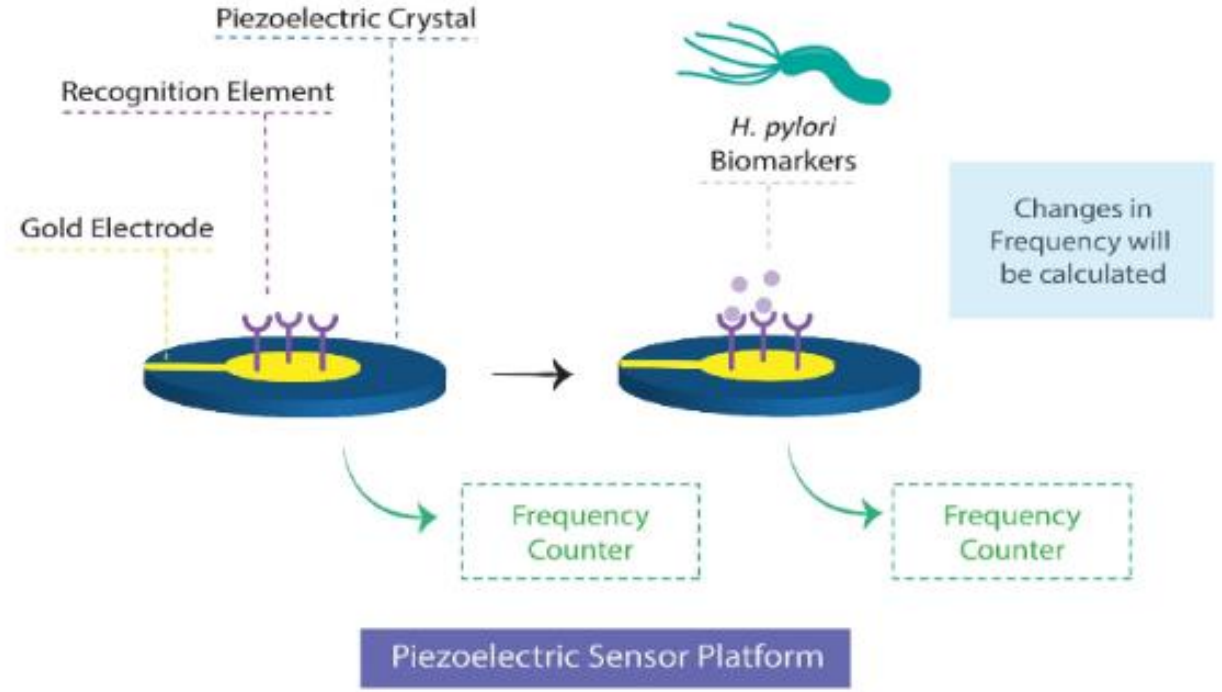


Fig. 4. A general schematic representation of piezoelectric biosensor for detection of *H. pylori* biomarkers.

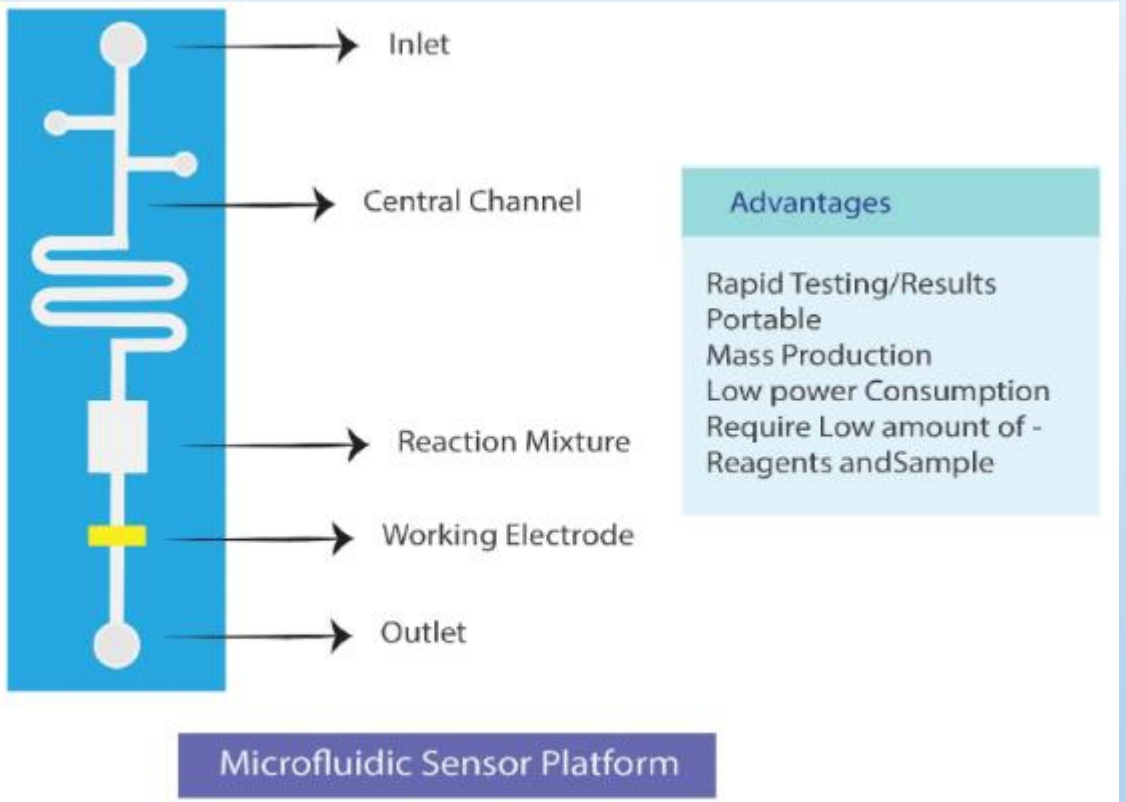
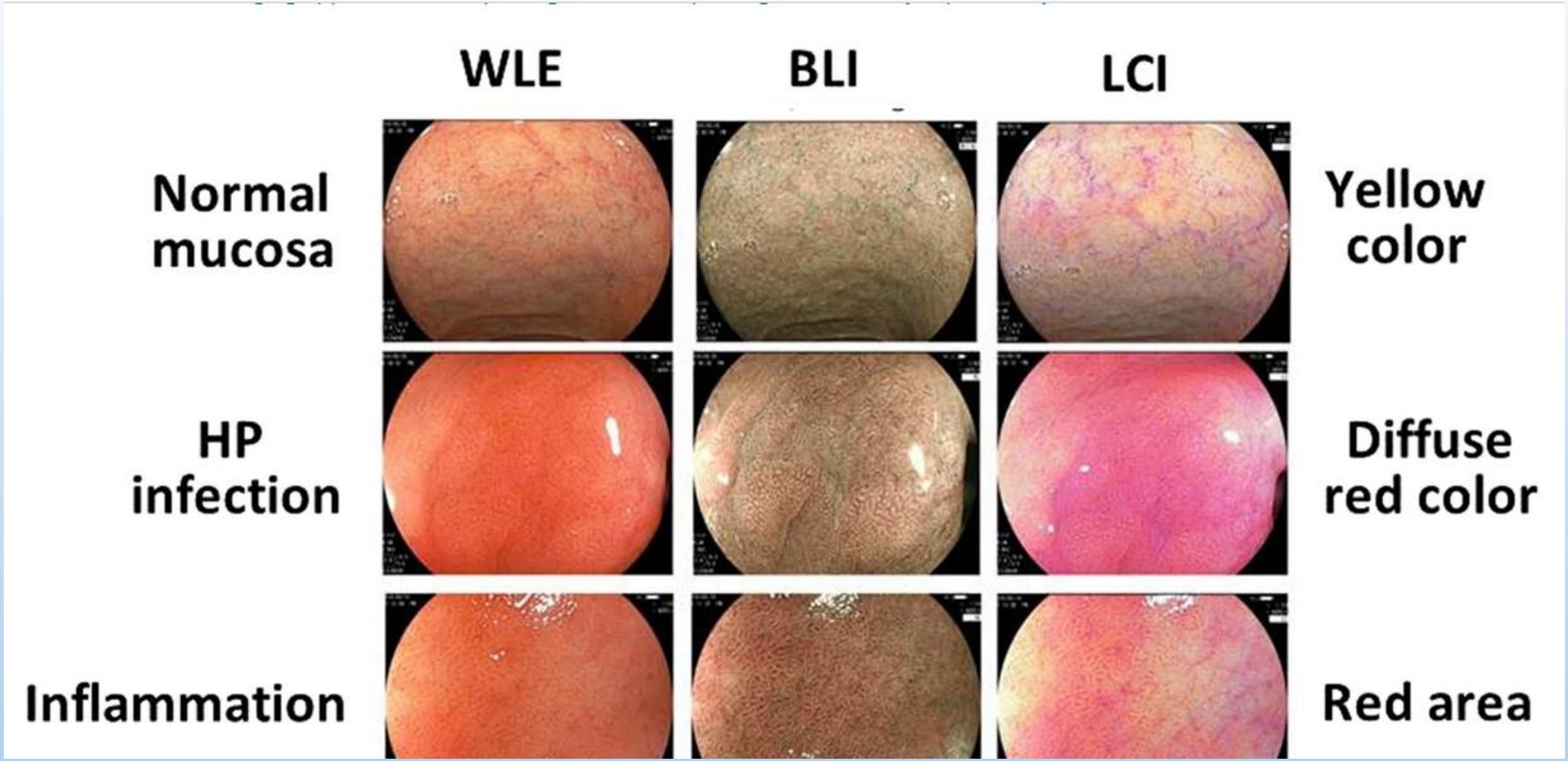


Fig. 5. A typical structure of microfluidic based bio-sensing platform.


WLE
BLI
LCI
Normal mucosa
Yellow color
HP infection
Diffuse red color
Inflammation
Red area


Thank you