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Biosensing technique for detection of H.pylori bacteria

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Abstract: ***H.pylori*** is an important cause of gastric duodenal disease, including gastric ulcers, Mucosa-associated lymphoid tissue (MALT), and gastric carcinoma. biosensors are becoming the most extensively studied discipline because the easy, rapid, low-cost, highly sensitive, and highly selective biosensors contribute to advances in next-generation medicines such as individualized medicine and ultrasensitive point-of-care detection of markers for diseases. Five of ten patients diagnosed with *H.pylori* ranging in age from 15–85 participated in this research. who [gastritis, duodenitis, duodenal ulcer (DU), and peptic ulcer (PU)] Suspected *H.pylori* colonies were identified by the presence of urease, catalase, oxidase activity, and PCR. All parameters are fixed: Laser power:40 mW, size of drops:25 µ, Turbidity:0.5. , Multi modes optical fiber, and Coreless optical fiber to construct optical biosensor (Multimode-Coreless-Multimode) optical fibers based on an inline Mach-Zehnder Interferometer. All samples had a sensitivity. Multimode-Coreless-Multimode optical Biosensor: is a rapid and sensitive method for the detection of *H.pylori* bacteria.

Keywords: Helicobacter pylori (H. pylori); Bacteria; bacterial detection; Optical Biosensor;

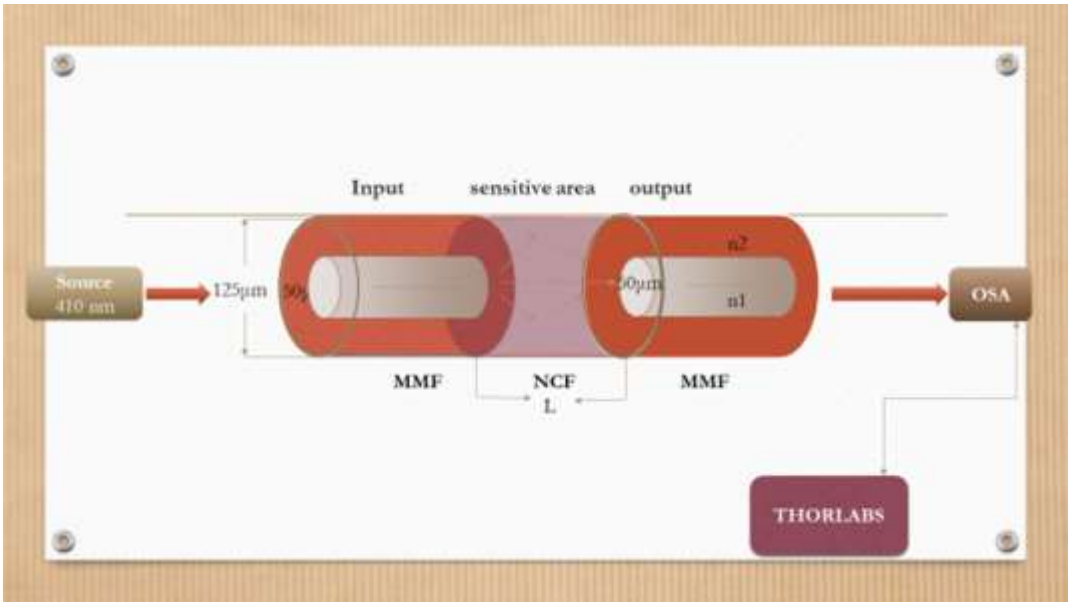
Introduction

H.pylori may infect the human stomach. It was first recognized in 1983 as playing a role in human illness. Bacteria are found in the stomach's inner wall and cause inflammation. infection Unless treated with eradication drugs, it seems to last a lifetime (Yamaoka and Graham, 2013). *H.pylori* infection always causes active chronic gastritis. This can be clinically overlooked throughout life in most people, but in a significant minority, gastroduodenal disease, especially digestive ulcer disease, cardia-free gastric cancer, and Mucosa-associated lymphoma tissue (MALT). To prevent and control bacteria in the environment, it is essential to create accurate, effective, and rapid methods for detecting bacteria (Cheng Y, 2017). During the last century, a range of methods for detecting bacteria was developed. Traditional culture methods, immunological techniques, molecular biology techniques, and biosensors are all types of these (Koyun, Ahlatcolu and Koca, 2012).Because of their proven high efficiency, biosensors are one of the most important technologies. Low cost, fast response, high sensitivity, and high selectivity (Ra *et al.*, 2012). Biosensors act by sensing target

molecules with biological materials (like antibacterial peptides, lectins, antibodies, and amplifiers, and signal processors are all examples of biosensor components (Pandey *et al.*, 2017). A detectable signal, such as an electrical or optical signal, is generated and detected by the transducer when a biological component detects an analyte and produces a catalytic or binding event (Usman *et al.*, 2019). The signal is proportional to the analyte concentration, Response time, dynamic range, detection limit, single-to-noise ratio, and specificity are all that affect biosensor performance. (Bobrinetskiy *et al.*, 2021). Biological detection elements, transducers, and signal amplifiers are all closely related to these elements. It is the most crucial part of biosensors (Monošík, Streďanský and Šturdík, 2012)Biosensors provide a fast and inexpensive way to detect bacteria and provide measurable or detectable readings. A biosensor is a method of visual, mechanical, or electrical means of communication and the receptors used (ie, catalyst [enzyme] or affinity-based [antibody, aptamer, lectin, bacteriophage]. Etc.), which can be categorized in different ways . In general, Biosensors have been developed for a variety of analytes, from single ions and small molecules to nucleic acids and proteins, viruses, and whole bacteria.This study aims to identify *H.pylori* isolated from Iraqi patients' clinical specimens before developing a new form. Using biosensors and their operating principles provides accurate, rapid, and practical detectors.

Materials and methods

In an ordinary method for isolation of *H.pylori* from patients, the sample was taken before proton pump inhibitor (PPI) ingestion of antibiotic therapy and directed endoscopy, and at least two simultaneous gastric mucosal biopsies were obtained approximately 3 cm from each patient-guided gastric duodenum. (One from the sinus and the other from the patient's stomach). Endoscopy (OGD) of the upper esophagus (OGD) under the supervision of an endoscopist with standard biopsy forceps using xylose as local anesthesia assesses the degree of clinical condition and A biopsy was performed immediately to determine the presence of aptamers) and generating detectable signals. Biological sensor elements, transducers, signal *H.pylori*. The culture is analyzed and processed as soon as possible, placed in the appropriate transport. Ideally, Within 6 hours for *H.pylori* isolation and identification. The biopsy was first ground with 0.5 ml burusera broth with an electric homogenizer. The suspension was then plated on a BHI broth medium. Columbia Blood Agar (10 mg / ml) enriched with 10% human blood and supplemented with vancomycin (10 mg / ml), ceftaroline (5 mg / ml), trimethoprim (5 mg- / ml) and action (100 mg / ml). After 24 hours in Himedia), the plates were incubated in a slightly anaerobic atmosphere (glass containing GasPaks) for 2 days. Suspected *H.pylori* colonies were identified by the presence of urease, catalase, oxidase activity, and PCR. In this work Multimode – Coreless –multimode (MCM) types of the optical biosensor are used to detect the *H.pylori* bacteria as shown in figure(1)based on inline Mach-Zehnder interference. The Multimode fiber (50 /125μm) core/cladding diameter and Coreless fiber (125μm) cladding diameter have the same procedure of fabrication. In which use optical fiber stripping tools to remove the coating for 2 cm long. The optical fiber stripper has three holes. The first hole is used for stripping the (1600-3000) μm, the second hole is used for striping the coating buffer of (600-900) μm, and the last hole is used to remove the acrylate coating of (250 μm) thickness. After removing the outer coating to obtain a flat edge of the fiber with 90 angles flat surface the cleaver machine(Fiber cleaver X-50C) By placing the fiber optic's edge on the cleavage machine's cutting blade, a perfectly smooth final edge is obtained. Use alcohol or a damp cloth to clean the fibers of the remaining coating. If the tip of the fiber does not cleave smoothly, the fiber needs to be cleaved again After we get a flattened edge of the fiber we use a Fusion splicing machine (Fusion splicer S-16)as shown in figure(2) MCM optical fiber 30cm in length was considered as the conventional optical fiber, a segment about 3 cm in length was made in the middle of the fiber using a cutter to make a .The whole fiber (30) cm was put on the plate using adhesive as shown in figure (3).



Figure(1): Fiber optic biosensor based on linked multimode no core multimode structure.

Figure(2): Fusion splicer S-16

The two ends of fiber were connected with an adapter device .An adapter is used for connecting the optical fiber with the laser from one side and the spectrum analyzer (OSA) from the other side.The source (410nm) was measured alone, then the turbidity of the samples was measured to 0.5 by a MacFarland standard to measure the intensity of *H.pylori* bacteria which detection by the biosensor.

Results and discussion

In this study, samples were taken from 10,five of it were positive.The isolation rate of *H.pylori* using Columbia blood agar was most of it positively as shown in figure (4) and it is also positive for urease as shown in figure (5) in addition to catalase, and oxidase-positive results as shown in figure (6) difference between each peak is the number of *H.pylori* cells in each drop.

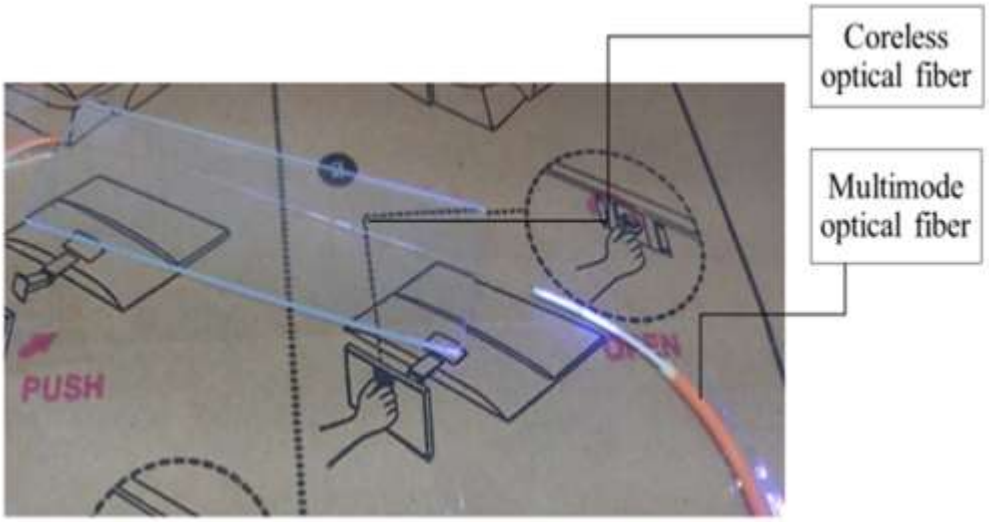
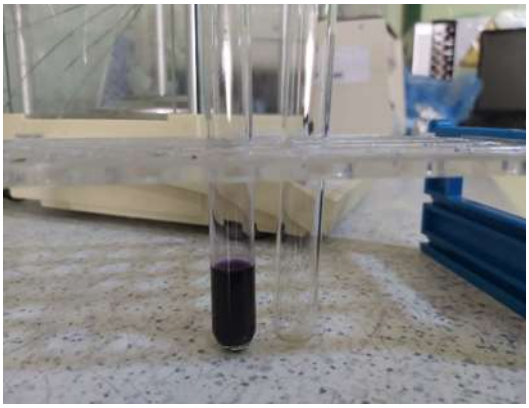


Figure (3) The Optical Biosensor Setup

Figure(4): h.pylori on selective Columbia blood agar culture

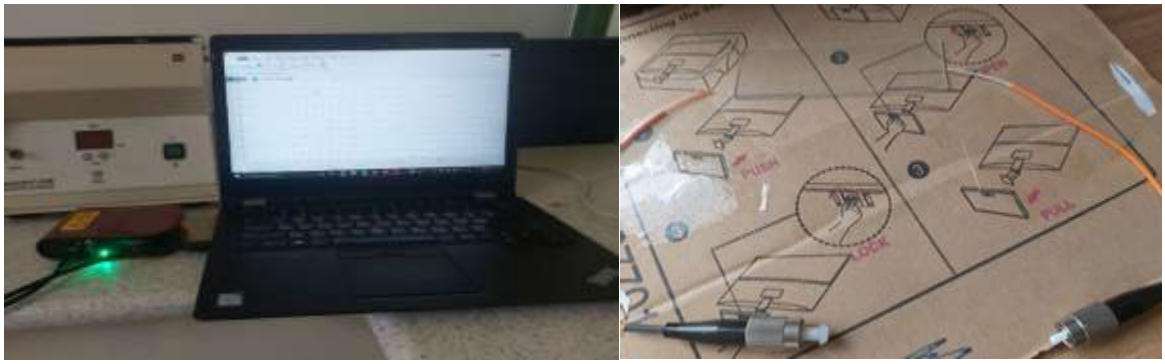
Figure(5) urease test agar



Figure(6): catalase, and oxidase test

Bacterial contamination of the medium by fungi and bacteria was common. The contaminating bacteria were Pseudomonas aeruginosa, the genus Proteus. Klebsiella can cause

contaminated biopsy forceps and contamination during collection, transport, and preparation of derailed sheep blood added to Columbia blood agar so the growth rate of H.pylori on this medium was slow and difficult to isolate so it was used the biosensor technique sensitive that produced rapid results for the determination of *Helicobacter pylori*. Flavin chemicals, together with porphyrins, as endogenous photosensitizers responsible for H. pylori photoinactivation have been identified for the first time in our spectroscopical and mass analytical analyses of bacterial extracts. Porphyrins, PPIX, CPI, and III, as well as flavin-type compounds like riboflavin, are among the endogenous photosensitizers responsible for bacterial photokilling.)Which absorb at 410 nm. after activating the samples for 24h, Broth media droplets (50 μm) are added to the coreless section(sensitive area).Detection is done by passing laser light from the source through the Multi-Mode area and passing it through the first splicing area. part of the light passing without reflection, while the other part is reflected from the splicing area to the coreless (sensitive area) to be absorbed by bacteria and reflected again to the second splicing area, which It collects light to pass through the multimodal region to the OSA (that inline Mach-Zhender interferometer)as shown in the figure(7)

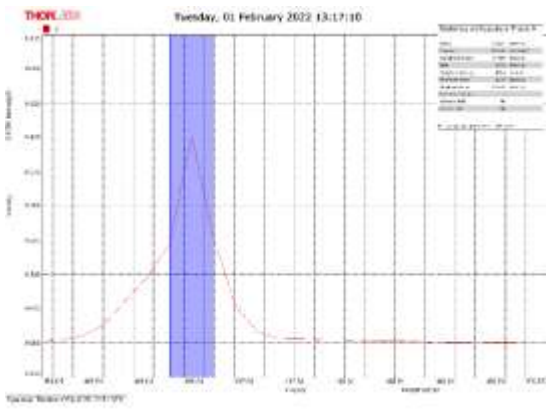


Figure(7): Biosensor setup in the lab

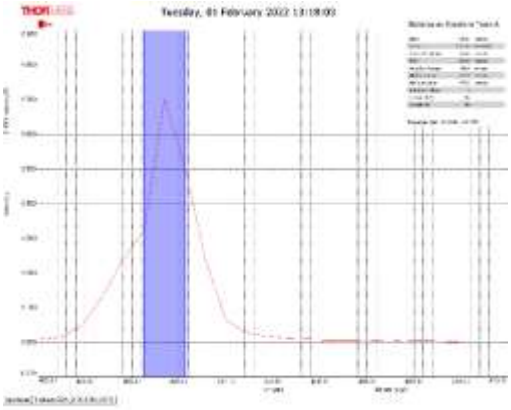
The Thorlab was showed a relationship between intensity and wavelength.Intensity is inversely proportional to the number of bacteria cells in the medea.The less the number of *H.pylori* bacteria the greater the intensity(Huang *et al.*, 2017) So Helicobacter pylori are measured as is shown in figures (8, 9, 10, 11, 12, 13). The time of the examination did not exceed half an hour, because *Helicobacter pylori* is an anaerobic bacteria, and thus the bio-sensor provided a safe, easy and fast method suitable with the conditions and sensitivity of bacteria. The results are comparable to previously reported multimodal interferometers based on more complex query schemes, and the MCM biosensor is a viable alternative for sensitive and easy measurements of H.pylori bacterial samples. Table (1) discuss the results where the

Table(1): Show results of culture, mean, variance, standard deviators, and range of wavelength in (nm).

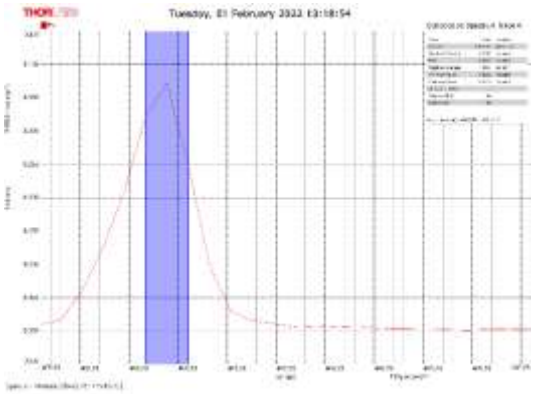
samples	culture	mean	variance	Standard deviators	Range(nm)air
1	+	0.3361	0.0274	0.1657	406.2-406.7 nm
2	+	0.5124	0.07255	0.2694	406.6-406.7 nm
3	+	0.347	0.0012	0.0351	406.2-406.7 nm
4	+	0.4146	0.03393	0.1842	406.2-406.7 nm
5	+	0.2756	0.0027	0.05258	406.0-406.5 nm
source		0.0993	0.0004433	0.02119	405.6-406.07nm



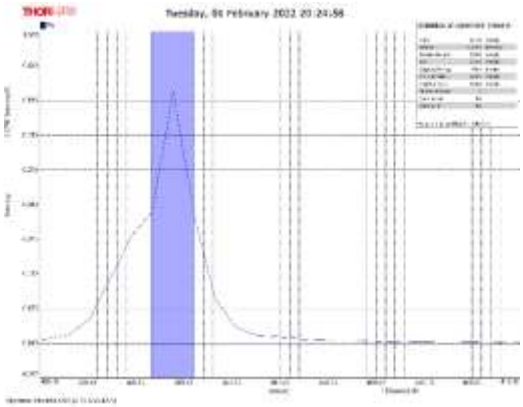
Figure(8): the peak of sample no.1



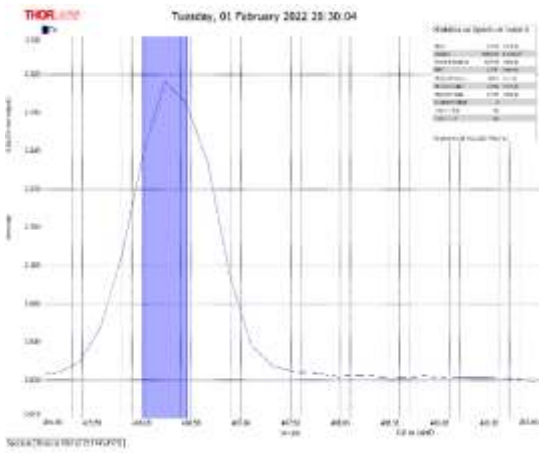
Figure(9): the peak of sample no.2



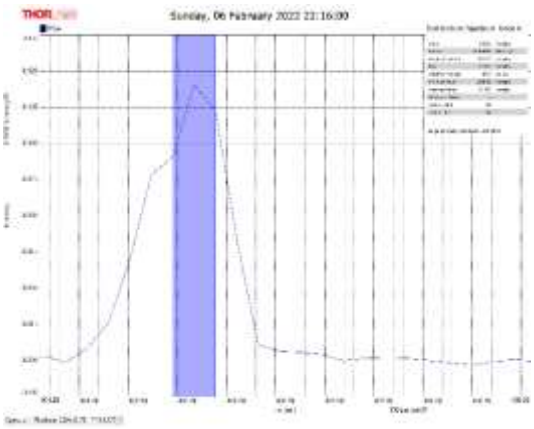
Figure(10): the peak of sample no.3



Figure(11): the peak of sample no.4



Figure(12): the peak of sample no.5



Figure(13): the peak of source

Conclusions

The isolation rate of *H. pylori* using Columbia blood agar was most of it positive. Bacterial contamination of the medium by fungi and bacteria was common. Our spectroscopical and mass analysis studies of bacterial extracts indicate for the first time flavin compounds, along with porphyrins as endogenous photosensitizers responsible for *H.Pylori* photoinactivation. The MCM biosensor was built based on the inline Mach-Zehnder interference is a viable alternative and rapid for sensitive and easy measurements of *H.pylori* bacterial samples. The results are comparable to previously reported multimodal interferometers based on more complex query schemes.All samples had a sensitivity ranging between 406.0 - 406.7 nm. Multimode-Coreless-Multimode optical Biosensor: is a rapid and sensitive method for the detection of *H.pylori* bacteria. (Statically) not all *H.pylori* isolates harbor the *Ure A* gene encoded for urease production

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تقنية الاستشعار الحيوي للكشف عن بكتيريا الملوية البوابية

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الخلاصه

الملوية البوابية هي سبب مهم لمرض الاثني عشر المعدية ، بما في ذلك قرحة المعدة ، والأنسجة اللمفاوية المرتبطة بالغشاء المخاطي ((MALT، وسرطان المعدة. أصبحت المستشعرات الحيوية أكثر التخصصات التي تمت دراستها على نطاق واسع لأن المستشعرات الحيوية السهلة والسريعة ومنخفضة التكلفة والحساسة للغاية والانتقائية للغاية تساهم في التقدم في أدوية الجيل التالي مثل الطب الفردي والكشف عن نقاط الرعاية فائقة الحساسية لعلامات الأمراض. شارك في هذا البحث خمسة من عشرة مرضى مصابين بالبكتيريا الحلزونية البوابية تتراوح أعمارهم بين 15 و 85 عامًا. من [التهاب المعدة ، والتهاب الاثني عشر ، وقرحة الاثني عشر ((DU، والقرحة الهضمية [(PU)تم التعرف على مستعمرات الملوية البوابية المشتبه بها من خلال وجود اليورياز ، والكتلاز ، ونشاط أوكسيديز ، و .PCRتم إصلاح جميع المعلمات: طاقة الليزر: 40 ميغاواط ، حجم القطرات: 25 ميكرومتر ، التعكر: 0.5. ، ألياف بصرية متعددة الأوضاع ، وألياف ضوئية عديمة النواة لبناء ألياف بصرية مستشعر حيوي ضوئي (متعدد الأوضاع - عديمة النواة - متعدد الأوضاع) استنادًا إلى مقياس تداخل Mach-Zehnder مضمن. جميع العينات لديها حساسية. المستشعر البيولوجي البصري متعدد الأوضاع - عديم النواة - متعدد الأوضاع: طريقة سريعة وحساسة للكشف عن بكتيريا الملوية البوابية.