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التشخيص البكتيري والمناعة الخلطية في المرضى المصابين بالملوية المعوية

رسالة
مقدمة إلى مجلس كلية العلوم / جامعة بابل
جزءا من متطلبات نيل درجة الماجستير في العلوم /علوم حياة

قدمها
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الخلاصة

الملوية المعوية هي بكتريا حلزونية حساسة للأوكسجين سالبة لصبغة الغرام وهي بكتريا تتسلل إلى داخل المعدة وتستقر في الخلايا الظهاريه في غشاء المعدة المخاطي قد تؤدي هذه البكتريا الى تكوين التهاب وتدمير في خلايا المتواجدة في غشاء المعدة وينتج عنها تقرح المعدة ولاثني عشر والتهابات مزمنة وفي بعض الأحيان الإصابة بسرطان المعدة على الرغم من الاستجابة المناعية لهذه البكتريا موضعيا وجهازيا الا أنها تستمر في البقاء ومقاومة المناعة لفترات طويلة قد تصل بعض الأحيان طيلة فترة حياة المضيف .

هدفت هذه الدراسة الى تشخيص هذه البكتريا وزراعتها ودراسة المضادات الحيوية وكذلك بعض المتغيرات المناعية مثل انترلوكين 10 و22 وبعض الغلوبينات المناعية مثل (IgA,IgE) .

شملت هذه الدراسة مائة وواحد وعشرين (121) عينة من أشخاص مرضى وأصحاء ، ممن يعانون من مشاكل في البطن ، وأعراض توحي بمرض القرحة الهضمية مثل آلام الجزء العلوي من البطن والحموضة والغثيان والقيء تم جمع ثلاث عينات مختلفة من الأشخاص بإشراف ومساعدة الطبيب والكادر المختص وبعد اخذ موافقة الأشخاص وهي (دم , براز , خزعة من المعدة) في مدينة الطب | بغداد مستشفى الجهاز الهضمي والكبد التعليمي وفي المستشفى العام لقضاء الصويرة | واسط للفترة من ايلول 2020 إلى نيسان 2021 حيث كانت عينات الذكور 31 عينة (25.61٪) وعينات الإناث 90 عينة (74.38٪) للمرضى والأصحاء .

تم جمع ثلاث عينات سريريه مختلفة (دم , براز , خزعة) وتم نقلها مباشرة داخل صندوق مبرد خلال فترة زمنية لا تتجاوز الثلاث ساعات الى مختبر الأحياء المجرية لإكمال العمل ألمختبري على العينات . تم عزل وتشخيص المرضى عن الأصحاء عن طريق فحص (اختبار الأجسام المضادة السريع في الدم) وأيضا (اختبار المستضدات السريع في البراز) وعن طريق التشخيص الطبي من خلال عمليات التنظير الداخلي في وحدة التنظير .

تم توثيق الإصابة بالبكتيريا الحلزونية في 71 مريضًا (58.67٪ من إجمالي 121 شخص) ، 21 (17.35٪) منهم من الذكور ، و 50 (41.32٪) من الإناث ، كانت نسبة الإناث أكثر مقارنة

الخلاصة

بالذكور في هذه الدراسة . في حين أن 50 (41.32٪) من الأشخاص كانوا أصحاء وفقًا لتشخيص الطبيب والتحليل ألمختبري ، واستخدموا كمجموعة السيطرة ، كان 10 (8.26٪) من الذكور ، و 40 (33.05٪) من الإناث.

تم إجراء اختبار الحساسية للمضادات الميكروبية عن طريق طريقة الانتشار القرصي على أجار مولرهنتن لسبعة مضادات حيوية تنتمي إلى خمس فئات. أظهرت النتائج وجود درجة عالية من المقاومة لمعظم المضادات الحيوية ، حيث كانت نسبة المقاومة أموكسيسيلين 65٪ ، دوكسيسيكلين 62.5٪ ، ميترونيدازول 100٪ ، ريفامبيسين 70٪ ، كلاريثروميسين ، أظهر مقاومة 30٪ وحساسية 65٪ ، سيبروفلوكساسين أظهر حساسية 65٪ بينما كانت نسبة المقاومة أكثر المضادات الحيوية فعالية وفقًا لنتيجة هذه الدراسة هو الليفوفلوكساسين الذي سجل أعلى حساسية (90٪).

تم استخدام تقنية الاليزا لتقدير وتقييم أربعة أنواع من المعلمات المناعية IL10 و IL22 و IgA و IgE ، حيث كانت نتائج IgA و IgE أعلى بشكل ملحوظ بين المرضى مقارنة بنتائج المجموعة السيطرة حيث كانت نتائج IgA 42.17 ± 92.59 في المرضى بينما كانت النتائج. 78.50 ± 18.70 في مجموعة السيطرة وكانت نتائج IgE 127.37 ± 325.73 في المرضى بينما كانت 285.98 ± 67.84 في مجموعة السيطرة وكذلك تم تسجيل فروق للفئات العمرية حيث لم تسجل المجموعة العمرية الثانية من 21 إلى 45 سنة فروق ذات دلالة إحصائية بين مجموعة المرضى ومجموعة السيطرة. تم تسجيل فرق معنوي بين الذكور والإناث ، حيث زاد في الإناث وينخفض عند الذكور. بالنسبة لنتائج الانترليوكين فيما يتعلق انترليوكين 10 ، لم نلاحظ فرقًا معنويًا بين المرضى ومجموعة السيطرة فقد كان 16.20± 27.60 في المرضى و 18.05 ± 30.40 في مجموعة السيطرة ، بينما كانت نتائج انترليوكين 22 هي 64.65 ± 84.90 في المرضى و 53.53 ± 54.94 في مجموعة السيطرة .

ظهرت علاقة إيجابية قوية بين IgE و IgA في مرضى الحلزونية البوابية. كما أظهر IgA ارتباطًا سلبيًا بالإنترلوكين -10 ، ولكن ارتباطًا إيجابيًا قويًا بالإنترلوكين -22. أيضًا ، أظهر IL-10 ارتباطًا سلبيًا مع IgE و IL-22 ، بينما أظهر IgE ارتباطًا إيجابيًا ضعيفًا مع IL-22.

Conclusion

1- The difficulty of isolating these bacteria from biopsy and identifying them and from feces, and the use of biopsy active media preserved immediately after sampling is very important for cultivation.

2- Initial identification of *H. pylori* can be based on three biochemical assays, catalase, oxidase and urease.

3- The topical strain of *H. pylori* has high resistance to most of the antibiotics used, especially amoxicillin and metronidazole, while levofloxacin is the preferred agent to eradicate these bacteria in this study.

4- There is a significant relationship between *H. pylori* infection and an increase in immunoglobulin (IgA, IgE) in the blood.

5- Both IL10 and IL22 have significant association with *H. pylori* infection.

Recommendation

1- Making bacteriological culture for biopsy for each patient introduce to GIT unit to identify suitable antibiotic.

2- Try other class of antibiotic in order to introduce more active drugs.

3 – Study other cellular .

Chapter One

Introduction

Chapter Two

Literature of Review

Chapter Three

Materials and Methods

Chapter Four

Results and Discussion

Conclusions and Recommendations

References

1-Introduction

Helicobacter pylori (H. pylori) is a Gram-negative microaerophilic bacterium that colonizes the human stomach and can caused to chronic gastritis, gastric, peptic ulcer adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. About half of the world's population is estimated to be infected with this pathogen (Zamani *et al.,* 2018).

H. pylori are small and 0.5-2 mm in diameter. In modern culture, bacteria appear in the form of a straight rod with an irregular curved rod, while in ancient culture they appear to be spherical in shape (Sofroniew *et al.*, 2001 ; Kimang'a *et al*., 2010). Infection is believed to play a important role in the path biology of gastric cancer , which is one of the most common malignancies with an estimated one million cases around the world in 2012(Park *et al.,* 2018). However, over 80 percent of individuals infected with this bacterium are asymptomatic (Hamzah and Aljanaby, 2020).

H. Pylori infection may be associated with the induction of various autoimmune disorders such as idiopathic rheumatoid arthritis thrombocytopenic purport (also called immune thrombocytopenic purpura) (Tanoue *et al*., 2019).

The route of transmission of *H. pylori* is known . The only known reservoir is the human stomach , new infections are thought to occur as a consequence of direct person-to-person transmission or by environmental contamination . It can be divided transmission in two main type: horizontal and vertical transmission. Horizontal transmission occur contact with individuals outside the family while the second the vertical mode is infection spread within same family (Stefano *et al*.,

2018).

H. pylori capable of creating a high level of urease into the stomach, which provides protection from gastric acidity. Urease produces NH_3 and $C0_2$ from urea and disrupts the epithelium by the production of ammonia. Ammonia interacts with Neutrophil metabolites, induces the formation of carcinogenic agents and therefore increase the risk of gastric cancer. Besides, urease induces production of inflammatory cytokines (Olive *et al*., 2014) .

Colonization of primary bacteria in gastric mucus depends on several factors including of the secretion bacterial urease enzyme (to resist acidic conditions of the gastric lumen), possession of polar flagella (for locomotion), and changes in bacterial cell morphology to effectively penetrate the gastric mucosa barrier and reach essential epithelial cells (Karkhah *et al*., 2019).

Among the cytokines produced by tolerogenic DCs, interleukin 10 (IL-10) is a key regulatory cytokine limiting und ultimately terminating excessive T-cell responses to microbial pathogens to prevent chronic inflammation and tissue damage (Schülke *et al*., 2018).

The Interleukin (IL)-10 cytokine family includes IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26, which are considered as Class 2α-helical cytokines. IL-10 is the most important cytokine in suppressing pro-inflammatory responses in all kinds of autoimmune diseases and limiting excessive immune responses. Due to protein structure homology and shared usage of receptor complexes as well as downstream signaling pathway, other IL-10 family cytokines also show indispensable functions

in immune regulation, tissue homeostasis, and host defense (Wei *et al*., 2019).

H. pylori has been shown to be one of the leading causes of peptic ulcer diseases (PUDs) and gastritis. T helper-22 (Th22) cells and its most important cytokine, interleukin-22 (IL-22) are importantly active in inflammation and inflammatory tissues. Since inflammation is one of the main attributes of infection caused by it and resulting complications (gastritis and gastrointestinal ulcer), this study was designed to evaluate the Th22 cells count and the IL-22 protein expression in people suffering from PUD and gastritis (Sanaii *et al*., 2019). Various cell types such as bronchial and intestinal epithelial cells, keratinocytes, hepatocytes, dermal fibroblasts, and tubular epithelial cells are affected by IL-22. Both pathological and protective roles are attributed to IL-22 in maintaining gut homeostasis and inflammation. IL-22 is highly involved in many diseases including allergic diseases, infection, autoimmunity and cancer development. Regulation of gut immune responses, barrier integrity, and inflammation depends on a variety of cytokines and mediators secreted by mucosal immune cells (Shohan *et al*., 2020).

Inflammatory cytokines in response to *H.pylori* disease result in the recruitment and development of certain T lymphocytes sensitive to *H. pylori* antigens and B cells that secrete immunoglobulins (IgA and IgG). However, the immune response cannot eliminate the bacteria unless antibiotic treatment is used, and the infection usually lasts for life (Ihan *et al*., 2012).

H. pylori is an important cause of global ill health due to its known etiological role in peptic ulcer disease, dyspepsia, gastric cancer, lymphoma, and more recognized in iron deficiency anemia and

idiopathic thrombocytopenic purpura. Increased antibiotic usage worldwide has led to antibiotic resistance among many bacteria, including *H. pylori*, resulting in falling success rates of first-line anti-*H. pylori* therapies. Eradication failures are principally due to resistance to clarithromycin, levofloxacin, and metronidazole. Several new treatment options or modifications of established regimens are now recommended by updated practice guidelines for primary or secondary therapy (Siddique *et al*., 2018).

The aim of study

Due to increase the prevalence of *H. pylori* infection, This study was conducted , to link the relationship between some Human immune variables and infection of the gastrointestinal tract from *H. pylori bacteria* through the following objective.

1- Rapid tests diagnosis to some GIT infection in different type of sample Blood , stool and biopsy .
2- Culturing stool and biopsy in appropriate media .
3- Antibiotic sensitivity of isolation bacteria.
4- Estimation some immunological parameters such as IgE . IgA .IL10 and IL22.

2- literature Review:

2-1 Historical review:

The history of these bacteria is inseparable from that of man. This is proven by genetic analyzes, which show that the bacteria most likely originated from East Africa and spread around the world about 58,000 years ago with the spread of modern humans. Studies show a relationship between breed diversity, human genetic diversity and geographical distribution. The first mention of *H. pylori* in the stomach dates back to 1875. George Butcher and Michael Letolle noticed these microbacterias at that time, but they were unable to cultivate them and perhaps because of this they did not present their discovery to the world In 2018, Marshall drank the bacterial suspension himself to prove himself that bacteria are the cause of stomach ulcers and gastritis. After a few days it struck nausea and a foul smell from his mouth, then vomiting appeared. The endoscopy results on the eighth day of the trial were clear - advanced enteritis (Fedorowicz *et al*., 2020) . Peptic ulcer disease had a clear interest in the mid-twentieth century and according to statistics, during the 1970s in the United States, there were more than 140,000 ulcer processes / year (Dandona *et al*., 2017).

In 1852, for the first time, ammonia was identified in the stomach and since that time it has remained a target of investigation and research . Medical devices designed to detect respiratory ammonia originally produced in the stomach are used clinically to detect Gram-negative bacterial disease. *Helicobacter pylori* infection is the most common causative agent of gastritis, ulcers, and stomach cancer. This discovery was

followed by widespread interest in infectious urease, including the group of animals in which urease can be found in addition to its role in health and disease (Graham & Miftahussurur, 2018).

The presence of *H. pylori* in the gastric mucosa was initially associated with an inflammatory process called gastritis, but most relevant from a clinical standpoint was the association of this bacterium with peptic ulcer disease (Ding *et al*., 2005) . This causative relationship was first identified by Steer in 1975 and followed by Warren in 1979 and was finally confirmed by Warren and Marshall in 1984 (Egan & O'Morain, 2007). Most diseases associated with *H. pylori* occurred in individuals older than 40 years of age. However, acquisition of *H. pylori* occurs mainly in young children inside the family setting (Li & Perez-Perez, 2018).

Robin Warren (pathologist at Royal Perth Hospital, Australia), noted for many years that had observed for many years that spiral-shaped gastric bacteria were a common finding in fresh samples obtained by endoscopy of patients with gastritis . However, although he was convinced that these bacteria were strongly linked to stomach diseases, he did not discuss them with the scientific community at that time, due to the prevailing idea that acidity was the main cause of peptic ulcer disease. Warren worked as a unit until 1982, after which he persuaded Barry Marshall, a resident physician who was looking for a research project, to help and work with him. In 1983, together, they successfully isolated and cultivated this bacterium that they called *Campylobacter pyloridis*. Years later it was called *Campylobacter pylori*, and since 1989, *Helicobacter pylori*. Late experiments have shown that these bacteria can colonize the human stomach and thus cause gastric mucositis (Tirado-Hurtado *et al*., 2019).

2-2 Classification of *H. pylori* :

Taxonomy aims to provide a meaningful biological framework upon which a wide range of other sciences is built. However, the inaccurate delineation of taxa serves to confound workers in a variety of disciplines (On, 2001). Cultivation of new stomach bacteria in 1982 marked a new turning point in our understanding of the environment and diseases of the digestive system. Description Marshall and Warren, spiral or curved bacilli in histological sections of numerous samples of the human gastric mucosa. A number of these samples gave culture positivity to the Gram-negative microorophilic bacteria. The second international workshop held in Brussels, Belgium, in September 1983 on *Campylobacter* infection gave great importance to its correct classification. The *Campylobacter* bacteria is similar in many aspects, including the curved morphology, growth on rich media under microrophilic conditions, failure to fermentation of glucose, sensitivity to metronidazole, and G + C content of 34%. Therefore it was first referred to as *Campylobacter pylori* (a doorman, or Greek, or gatekeeper, or a person looking in both directions) and was verified as *Campylobacter* in 1985 . The specific trait was revised to *Campylobacter pylori* in 1987 to correspond with the correct Latin additive for the name pylorus (Solnick & Vandamme, 2001) .

Domain *Bacteria*

Phylum *Proteobacteria*

Class *Epsilonproteobacteria*

Order *Campylobacterales*

Family *Helicobacteraceae*

Genus *Helicobacter*

2-3 General Characteristics and Growth Requirement of *H. pylori*

Helicobacter pylori is a Gram-negative bacterium that chronically infects the stomachs of 50% of the human population. *H. pylori* infection is a significant risk factor for the development of peptic ulcer disease, gastric adenocarcinoma, and gastric lymphoma . One of the important virulence factors expressed by this bacteria is VacA, a secreted toxin named for its capacity to induce extensive vacuolation in the cytoplasm of mammalian cells (Gangwer *et al*., 2007).

A discovery, gram negative, microaerophilic bacterium *Helicobacter pylori* colonizes the human gastric mucosa and establishes a chronic infection that is tightly associated with atrophic gastritis, peptic ulcer, and gastric carcinoma. *H. pylori* infection is a worldwide problem, since in developing countries it affects over 80% of the population older than 20. Also in developed countries the infection is present in 20% of the population by the age of 30 rising to over 50% by the age of 60. Clinical isolates of *H. pylori* can be classified into two groups based on the presence or absence of the vacuolating cytotoxin (Cover & Blaser, 1992).

H. pylori has a unique helical cell shape and fine-tuned flagellar motility . Compared to other motile bacteria, *H. pylori* cells have the unique ability to swim rapidly within the viscous mucosal layer of the stomach . It has become increasingly clear that this unique motility is essential for its successful gastric colonization and pathogenesis . The motility of *H. pylori* is driven by multiple unipolar flagella, which are enveloped within a membrane sheath. The overall organization of the

H. pylori flagellum is similar to those of the model bacterias *Escherichia* coli and *Salmonella*, which have been extensively studied and comprehensively reviewed . The *H. pylori* flagellum is composed of the following three major parts: a rotary motor embedded in the cell envelope, a filament that functions as a helical propeller, and a hook serving as a flexible joint between motor and filament. At least several dozen different proteins are involved in the assembly and function of the polar flagellum (Qin *et al.*, 2017).

During infection of the stomach, *H. pylori* largely has an actively dividing, spiral-shaped (helical) morphology. However, upon entry into the intestinal tract, *H. pylori* transitions to a viable but not culturable coccoid form to enable it to contend with the unfavorable anaerobic conditions there. This transformation has also been observed under adverse conditions, such as in spent cultures, aerobic conditions, and in the presence of antibiotics The coccoid form is not culturable by standard laboratory methodsin vitro. However, it has been postulated that these cells are viable and possibly even infectious and that the coccoid form is a dormant stage that allows *H. pylori* to survive hostile environments (Hirukawa *et al*., 2018).

H. pylori is small in size and the diameter range between 0.5-2 mm. in the recent culture the bacteria appear in rod straight form with an irregularly curved rod, while in the old culture it seems to be spherical in shape . *H. pylori* is characterized by the fact that they need a small amount of O_2 reaching to 5-10% and need Co_2 10% and the suitable amount of pH, which is 7 . *H. pylori* usually requires 4-7 days of incubation for the small translucent initial circular colonies to develop.

H. pylori is typically diagnosed according to cellular morphology and positive oxidase reactivity, and by catalase and rapid-urease test results (Al-Thahab & Al-Awsi, 2018).

H. pylori has been demonstrated to grow well in several nutrient-rich media, such as chocolate media, brucella media, Columbia media, and brain heart infusion (BHI) media (Lee *et al*., 2017) .

The standard treatment for *H. pylori* infection is called triple therapy that consists of two antibiotics (e.g. clarithromycin and amoxicillin) and a proton pump inhibitor such as omeprazole (Malfertheiner *et al*., 2007). The emergence of antibiotic-resistant *Helicobacter pylori* has become a problem, which has reduced the rate of successful eradication of *H. pylori*. However, The use of different types of antibiotics (such as metronidazole) and a new inhibitor of potassium acids (phonoprazan) has significantly increased the eradication rate above 90% or more (Suzuki *et al*., 2016).

2-4 Transmission:

During the first years of life, *H. pylori* infection is usually acquired, the mode of transmission is unknown, although several authors are thought to occur through direct human to human communication. In most cases, it occurs through the fecal oral routine or oral-oral or gastro-oral routes within families (Aziz *et al*., 2015) . The transmission route of *H. Pylori* is not well known . The only reservoir that is known is the human stomach (Schwarz *et al*., 2008) and because *H. Pylori* tends to have a limited host range, as a consequence of direct human-to-human transmission or environmental contamination, new infections are thought to occur.

Transmission from person to person can be subdivided into two major categories: vertical and horizontal transmission .The vertical mode is an infection transmitted within the same family from ascendant to descendant, while horizontal transmission includes interaction with people outside the family or contamination of the environment (Stefano *et al*., 2018). *H. Pylori* is acquired early in life, and achlorhydric mucus vomiting can serve as a transmission vehicle. The transmission route may be through gastric juice, especially as a consequence of childhood vomiting (Bürgers *et al*., 2008) . Another potential source of *H. Pylori* may be saliva, since, after regurgitation or vomiting, the gastric micro biome can reach and colonize the mouth. *H. Pylori* has been cultivated directly from saliva, and saliva, subgingival biofilm and dental plaque have also amplified the DNA (Gebara *et al*., 2006).

2- 5- Virulence factors:

Helicobacter pylori is a bacterium that has infected more than half of the human population worldwide. This bacterium is closely associated with severe human diseases, such as gastric cancer, and recognizing and understanding factors that predict bacterial virulence is a priority. Furthermore, this pathogen displays a high degree of genetic variation and co-evolution with its human hosts (Kabamba *et al*., 2018). There are several virulence factors in *H. pylori* that affect colonization and disease severity. Some of these factors contribute to its long-term survival. Urease is present in all clinical isolates of *H. pylori*. Urease converts to ammonia plus carbon dioxide . Raising the pH of the surrounding atmosphere. This offers temporary stomach acid defense, but *H. pylori* is not an acidophil.

The near-neutral pH needed found immediately adjacent to the gastric surface epithelium in the mucus coat. The helical form of *H. pylori* makes it easier to propel *H. pylori* through the viscous mucus of its polar flagella (Lertsethtakarn *et al*., 2011; Testerman & Morris, 2014). The strains of *H. pylori* are divided into two subpopulations on the basis of the capacity to generate CagA: cagA-positive and cagA-negative. A greater degree of gastric inflammation is associated with the cagA-positive *H. pylori* strains and is more virulent than the cagA-negative strains (Iunusova *et al*., 2017).

H. pylori has an effect on stomach cancer. The evolution of *H. pylori* is associated with the gene A (CagA) linked to cytotoxin, which is injected into the stomach-lining epithelial cells. Both C and N-termini for CagA interact with a number of host proteins, causing disruption of essential cellular functions including cell adhesion, cell cycle, and intracellular signaling and the cytoskeleton structure. CagA's N-terminus interacts with the tumor suppressor protein apoptosis-inducing protein p53 (ASPP2), inactivating the p53 tumor suppressor gene's apoptosis feature (Junaid *et al*., 2018). CagA is a well-recognized oncoprotein which is injected into host cells via a pilus structure called type IV secretion system (T4SS) (Odenbreit *et al*., 2000).

Since the discovery of VacA nearly 25 years ago as the proteinacious factor within *H. pylori* culture filtrates that intoxicates epithelial cells and induces vacuole biogenesis (Leunk *et al*., 1988). The vacuolating cytotoxin (VacA) secreted by *H. pylori* is an 88 kDa protein with two important p33 and p55 subunits. The p33 in the N-terminal of protein forms an inner channel for chloride transport and the p55 in the N-terminal of protein is indispensable for binding of the. Following internalization, VacA

accumulates inside different cellular compartment and induces apoptosis. Moreover, VacA can cause the dissipation of mitochondrial transmembrane potential , cytochrome c release, and activation of pro-apoptotic factor Bcl-2 associated X protein (Bax) by transferring to mitochondria and, thereby resulting in apoptosis . (Nejati *et al*., 2018). Vacuolating cytotoxin A (VacA), a major toxin facilitating the bacteria against the host defense system. The toxin causes multiple effects in epithelial cells and immune cells, especially T cells, B cells, and Macrophages (Chauhan *et al*., 2019).

Lipopolysaccharides LPS is an important cellular component of the outer membrane of Gram-negative bacteria, and has three distinct components: the core oligosaccharide, the lipid-A region, and the O-antigen (Kusters *et al.*, 2006) . lipopolysaccharides (LPS) are an important cellular component of the outer membrane of *H. pylori*. The LPS of this bacteria plays a key role in its colonization and persistence in the stomach. In addition, *H. pylori* LPS modulates pathogen-induced host inflammatory responses resulting in chronic inflammation within the gastrointestinal tract (Leker *et al*., 2017) .

2-5- Adhesion:

Bacterial adhesions are cell surface proteins that enable bacteria to bind to cells. The first step in pathogen colonization and pathogenesis is pathogen adhesion to mucosal epithelial cells. *H. pylori* adhesion to the gastric mucosa is critical for defense against processes such as acidic pH, mucus, and exfoliation. *H. pylori* adhesions are bacterial virulence factors that play a role in a variety of processes during the early and late stages of infection. They also play a role in the different outcomes of infected

patients by causing disease to develop. *H. pylori* adhesive factors are members of the bacterium's largest outer membrane protein (OMP) family, the Hop family (Mueller *et al.*, 2012) .

2-6- Diagnostic Techniques:

(H. pylori) resides in the stomach, colonizes gastric epithelium, and causes several digestive system diseases. Several diagnostic methods utilizing invasive or non-invasive techniques with varying levels of sensitivity and specificity are developed to detect *H. pylori* infection. Selection of one or more diagnostic tests will depend on the clinical conditions, the experience of the clinician, cost, sensitivity, and specificity. Invasive methods require endoscopy with biopsies of gastric tissues for the histology, culture, and rapid urease test. Among non-invasive tests, urea breath test and fecal antigen tests are a quick diagnostic procedure with comparable accuracy to biopsy-based techniques and are methods of choice in the test and treatment setting. Other techniques such as serological methods to detect immunoglobulin G antibodies to *H. pylori* can show high accuracy as other non-invasive and invasive biopsies, but do not differentiate between current or past *H. pylori* infections. Polymerase chain reaction (PCR) is an emerging option that can be categorized as invasive and non-invasive tests. PCR method is beneficial to detect *H. pylori* from gastric biopsies without the need for the cultures (Sabbagh *et al*., 2019) . It can also be diagnosed by RT PCR (Peng *et al*. 2017) .

2- 6-1- Method based on discovery of the enzyme:-

2-6-1-1- Rapid urease test (RUT)

The fact that *H. pylori* is both abundant in the stomach and contains urease has been widely used to assist in clinical diagnosis., with a short time after the discovery of *H. pylor*i, rapid urease tests had been developed to allow rapid detection of *H. pylori* using gastric specimens (mucus, biopsy, or brushings)., methods to detect changes in pH either directly or using color changes following incubation of gastric specimens proved both simple and reliable and were most widely adapted (Graham & Miftahussurur, 2018).

Biopsy samples, approximately 2–3 mm each were taken from the antralgastric mucosa and placed on the yellow colored well containing urea and a pH indicator. The production of the urease enzyme by *H. pylori* results in the decomposition of urea into bicarbonate and ammonia which causes the pH to rise and the colour of the dot to change from yellow to red or pink. Positive results were read within 5 to 30 min. Samples that were weakly positive took up to 1 h to develop and no colour change at 1 h was regarded negative (Adu-Aryee *et al*., 2016).

2-6-1-2- Urea breath testing (UBT)

The urea breath test is a non-invasive, simple and safe test which provides excellent accuracy both for the initial diagnosis of *Helicobacter pylori* infection and for the confirmation of its eradication after treatment.Some studies have found no differences between urea breath test performed under non-fasting conditions. The simplicity, good tolerance and

economy of the citric acid test meal probably make its systematic use advisable. The urea breath test protocol may be performed with relatively low doses (<mg seem to be sufficient. With the mg or even 50 mg) of urea: 75 100 mg of urea), excellent most widely used protocol (with citric acid and 75 min accuracy is obtained when breath samples are collected as early as 10–15 minute after urea ingestion (Gisbert & Pajares, 2004 ; Lopes *et al.*, 2014) .

Several factors including the patient's condition, bacterium, and the test itself can affect the results of the UBT . Nonetheless, the urea breath test is widely available because breath samples are easy to collect for rapid testing . UBT is useful for epidemiological studies and for assessing the effectiveness of eradication therapy . This method has advantages such as non-invasive, safe, accurate, and with a sensitivity of 95.9% and a specificity of 95.7% (Sabbagh *et al*., 2019).

2-6-2- Method based on discovery of antigen-antibodies to *H. pylori*

2-6-2-1- Stool antigen test

In 1997, it was reported that the detection of *H. pylori* antigens in stools using polyclonal anti-*H. pylori* antibodies (HpSA) with a sensitivity and specificity of 88.8% and 94.5%, respectively (Mégraud & Lehours, 2007 ; Miftahussurur & Yamaoka, 2016).

In infected individuals, *H. pylori* sticks to the gastric epithelial wall and is excreted in the feces. This test is a direct test of initial infection that results in the superiority of serologic tests . The test is based on the

detection of *H. pylori* antigens in the stool. There are two types of SATs used for *H. pylori* detection: enzyme immunoassay (EIA)– and immunochromatography assay (ICA)–based methods, using either polyclonal antibodies or monoclonal antibodies . Monoclonal antibody–based tests show better results compared to polyclonal-based tests mainly because of the difficulty in obtaining polyclonal antibodies of consistent quality every time. EIA-based tests provide more accurate and reliable results than ICA-based tests although both tests can be performed with monoclonal antibodies (Thaker *et al*., 2016 ; Sabbagh *et al.*, 2019).

2-6-2-2-*H.pylori* specific antibodies

Serological testing is the most widely available test for the detection of *H. pylori* with a relatively high negative predictive value . Furthermore, serology is the only test that is not affected by local changes in the stomach that could lead to false-negative results in the other tests. Furthermore, in patients treated with PPIs, if it not possible to stop them for at least 2 weeks, a validated IgG serology test (ELISA) may be used (Tonkic *et al*., 2012).

This type of testing is not appropriate for proving current infection since IgG antibodies are present for such a long period during a patient's life. It can't tell the difference between past and current infections. Serology cannot be used to track eradication for the same purpose. Antibodies will last a lifetime after eradication. (Miftahussurur & Yamaoka, 2016).

2-6-3- Method based on the presence of bacterium

2-6-3-1-Culture

Several selective media are suggested for *H. pylori* culture and isolation. The transport medium, time in transit to the pathology laboratory, temperature during transportation, and the medium used all influence bacterial viability and recovery; all of these factors influence bacterial viability and recovery (Graham & Miftahussurur, 2018).

The culture of *H. pylori* is performed on the gastric biopsy samples to confirm the *H. pylori* infection and is performed only in specialized laboratories . Culturing of gastric biopsy samples to detect *H. pylori* is not a routine method for detecting *H. pylori* . Bacterial culture is carried out mainly for scientific research and when the prior treatments have failed to detect an appropriate bacterium. It is recommended that the mentioned test should be performed before the next treatment line to determine the microbial susceptibility (Sabbagh *et al*., 2019).

Although the culture is an expensive, complicated, and time-consuming test for *H. pylori* detection, an antibiotic susceptibility test of *H. pylori* by culture is a useful clinical practice for accurate detection. Besides, culture allows the isolation of *H. pylori* for phenotypic and genotypic studies (Wang *et al*., 2015) .

2-6-3-2-Endoscopy

A typical endoscopy exam is performed to detect *H. pylori*–related diseases. Endoscopy is the method for obtaining biopsies from the gastric mucosa that can be used in further studies on other invasive methods (Lan *et al.*, 2012) . Also, the endoscopy offers the precise and clear image of gastric mucosa, but it may not have better results than other diagnostic tests (Cho *et al*., 2013). The major disadvantages of this procedure include the time-consuming process and require a lot of skill and (Sabbagh *et al.*, 2019).

2-6-3-3- PCR for *H. pylori* Markers

The polymerase chain reaction (PCR) is an effective molecular technique that can be used in a number of clinical settings, including broad-spectrum infection detection, emerging infection assessment, genotypic bacterial identification, antibiotic resistance, and epidemiological studies (PCR) tests are used for diagnosis. PCR detection of *H. pylor*i has been reported using a variety of clinical samples including gastric biopsy, gastric juice, saliva, dental plaque, and stools as well as environmental samples. Whenever possibly, noninvasive tests are preferred over invasive tests. *H. pylori* are excreted in the stool. Culture from stool is variable whereas stool antigen testing is widely used. Stool consists of a complicated mixture of communal bacteria and chemicals and often includes inhibitors of PCR. Nevertheless, simple extraction methods are available to efficiently extract DNA from human stools and nested-PCR targeting the 23S rRNA gene have proven to be highly sensitive for the detection of *H. pylori*. Detection of clarithromycin susceptibility/resistance is important clinically and the

mutation of the 23S rRNA gene responsible for resistance can also be detected using stool. This described method can be modified for other clinical samples such as gastric juice or biopsy material (Rimbara, *et al*., 2013).

Antibiotic resistance mutations can be detected using molecular techniques such as PCR, which is an appropriate methodology for pathogen detection and can assist us in selecting an appropriate treatment strategy (Ramírez-Lázaro *et al*., 2011) .

2-7- Immune Response to *H. pylori*

H pylori is a bacterium that colonizes the human stomach and causes inflammation, which can lead to gastric ulcers and cancer in some cases. A dynamic interplay of bacterial, host genetic, and environmental factors influences the clinical outcome of infection. Despite the fact that both the innate and adaptive immune systems accept *H. pylori*, bacterial clearance is uncommon. The first line of protection against *H. pylori* is gastric epithelial cells, which alert the immune system to the presence of the bacteria (Gall *et al*., 2017).

H. pylori has co-evolved with humans for at least thousands of years, and chronic *H. pylori* infection was most likely the "natural" human condition in pre-modern times. As a result, it appears that *H .pylori* this is thought to help them survive and contribute to their remarkable stability throughout their lives. However, in disease, the inflammatory component of the response is crucial. Other aspects of the local and systemic response are also essential to pathogenesis, according to mounting evidence (Robinson *et al*., 2007).

2-7-1- Innate immune response

The gastric bacterium *H. pylori* efficiently evades innate immune detection and persistently colonizes its human host (Zhang *et al*., 2020).

H. pylori has long been known to activate canonical NF-kB(Nuclear factor kappa B) signaling in gastric epithelial cells through mechanisms that depend on the bacterium's Cag pathogenicity island-encoded type IV secretion system (T4SS) (Backert & Naumann, 2010) . NF-kB regulates the expression of chemokines and cytokines involved in immune cell recruitment, as well as epithelial cell-intrinsic innate defense mechanisms. Microbial pathogen-associated molecular patterns (PAMPs) or pro-inflammatory cytokines such as TNF-α cause NF-kβ signaling (Newton & Dixit, 2012).

The levels of IL-12 and IL-2 steadily decreased during the progression of *H. pylori* infection-related pathologies, but IL-10 increased, indicating a shift in cellular to humeral immunity patterns. A polarized type 2 immune response, but not a type 1 immune response, has been found to be more common in chronic gastritis, precancerous lesions, and cancer . and gastric adenocarcinoma caused by *H. pylori* infection (Li *et al*., 2017).

Innate lymphoid cells (ILCs) are distributed in a wide variety of epithelial compartments and act as an intermediate position between acquired immune cells and bone marrow cells. Based on their cytokine production patterns that correspond to the T helper cell subsets Th1, Th2, and Th17, ILCs are commonly classified into three groups: ILC1, ILC2, and ILC3, respectively (Moro & Koyasu, 2015) .

Other studies have shown that group 2 ILCs (ILC2s) are the dominant subtype in the stomach of both humans and mice. Stomach ILC2s are unique in that their existence is dependent on stomach microbiota, in sharp contrast to the lack of an impact of commensal microbiota on ILC2s in other tissues. The microbiota dependency of stomach ILC2s is partly explained by their responsiveness to interleukin (IL)-7. Stomach ILC2s express significantly higher IL-7 receptor protein levels on their surface and proliferate more in response to IL-7 stimulation in vitro than small intestinal ILC2s. Consistently, the stomach expresses much higher IL-7 protein levels than the small intestine. IL-5 secreted from stomach ILC2s promotes immunoglobulin (Ig) A production by plasma B cells. In a murine model, stomach ILC2s are important in containing *Helicobacter pylori* infection, especially in the early phase of infection, by promoting IgA production (Ohno & Satoh-Takayama, 2020) .

2-7-2-Cellular immune response

Autophagosome formation is induced by *H. pylori* infection and these autophagic vesicles are adopted for replication of *H. pylori* and subsequent eradication of the invading *H. pylori* in macrophages. Some Taiwanese clinical isolates of *H. pylori* can replicate in certain macrophage cell lines. After entry, there was a 5-10 fold increment of re-cultivable *H. pylori* from the infected permissible cells at 12 h post infection. The dividing *H. pylori* are observed to reside in double-layered autophagosomes. Therefore, *H. pylori* can be considered as a kind of intracellular microbacteria. The autophagy induction by *H. pylori* is not only found in macrophages, but also in dendritic cells and gastric epithelial cells. This new finding has several implications for the life cycle of *H. pylori* in the

host. The bacterium's residence inside infected cells will increase its resistance to antimicrobial treatment, avoid neutralization by anti-*H. pylori* antibodies, impair antigen presentation, and alter the cellular immune response. The replication of *H. pylori* in autophagic vesicles, and the consequences of this provide an important hint as to why this microbacteria causes so such a broad spectrum of diseases.(Wang *et al.*, 2009). *H. pylori* could multiply not only in macrophages, but also in dendritic cells and epithelial cells. This new finding has several implications for the life cycle of *H. pylori* in the host. *H. pylori* can be considered as a kind of intracellular microbacteria because it can invade host cells to undergo replication within the autophagosome. The bacterium's residence inside infected cells not only increases its resistance to antimicrobial treatment, but also allows it to avoid immune attack by humoral antibodies, and so this aspect will arguably influence our understanding of how the immune system responds to orally ingested *H. pylori*. Infection with *H. pylori* induces vigorous antibody and cellular immune responses at both systemic and mucosal levels. However, these immunities are not protective. The question arises as to why the anti-*H. pylori* immune response is not adequate. For bacterial infections, the antibody with opsonization activity (ability of an antibody Fab domain to bind a pathogen and Fc domain to bind a receptor on a phagocyte) and phagocytes are major effectors to clear the bacterium (Harris *et al*., 2000).

Epidemiological evidence suggests a relationship between *H. pylori* infection with the development of autoimmune diseases. *H. pylori* elicit a chronic systemic inflammatory response with the secretion of proinflammatory cytokines. IL-10 is a regulatory cytokine that plays a

central role in limiting host immune response to pathogen. Increased IL-10 levels were reported in *H. pylori*–infected gastric mucosa (Yamamoto-Furusho *et al*., 2020). Interleukin 10 (IL-10) is a cytokine implicated in the regulation of human intestinal immune responses. This cytokine acts such as one of most important regulatory interleukins acting in antigen-presenting cells through the inhibition of cytokines and HLA class II (Owyang *et al*., 2012).

H. pylori bacteria stimulate an inflammatory response both in the gastric epithelial cells and the immune cells recruited to the site of infection through multiple mechanisms.It has been shown that the *H. pylori* infection up regulates the functional expression of several pro-inflammatory cytokines such as interleukin (IL) 1, IL-6, IL-8, tumor necrosis factor α (TNF-α), NF-κβ, and also regulates the activation of regular T cell . These cytokines, particularly NF-κβ, are key mediators of gastric path physiology and may perform important roles in the development of gastric inflammation and cancer (Lamb & Chen, 2013).

IL-22 was identified in 2000 in the secretome of IL-9-stimulated thymic lymphomas and it was initially named “IL-10-related T cell-derived inducible factor” (IL-TIF) since it has roughly 22% protein sequence homology to mouse IL-10 (Dumoutier *et al*., 2000). IL-22 is now largely seen as a cytokine secreted by lymphoid cells, such as group 3 innate lymphoid cells (ILC3), CD4+ T cells, γδ T cells and CD1d-restricted T cells (a.k.a., iNKT cells) . Few reports have found cells of the myeloid lineage (such as neutrophils) to produce IL-22 (Zindl *et al*., 2013).

2-8-*H. pylori* Associated Diseases

2-8-1-Gagster cancer

H. pylori infection almost doubles the overall risk of gastric cancer. GC develops through a cascade of well-defined and well-recognizable steps: inflammation, atrophy, intestinal metaplasia, dysplasia, and carcinogenesis, and is closely associated with the environment, diet and gene mutations (Herrera & Parsonnet, 2009). Cytotoxin associated factor (CagA) and vocalizing cytotoxin (VacA) are specific gene of *H. pylori*, which are mainly virulence factors involved in increasing the risk for gastric carcinoma development . CagA - protein is a 120- to 140-kDa protein that is translocated into host cells by the type IV cag secretion system, after the attachment of *H. pylori* and in the result changes the cell-signalling mechanisms in gastric cells. VacA protein is a cytotoxin produced by bacteria and inducing vacuolation of the epithelial cells. The gene is present in all strains but has different variations of vacuolating activity (Matos *et al.,* 2013 ; El Khadir *et al.,* 2017).

At a gene level, hypoxia-inducible factor 1 (HIF-1) is the primary transcriptional activator, very sensitive to oxygen, helping cells to survive in low oxygen tension . Over expression of HIF-1α is important in the activation the bunch of genes involved in cancer biology, encompassing cell proliferation, survival and apoptosis, glucose metabolism, erythropoietin, as well as angiogenesis . It was displayed that the expression of around 20 genes is regulated by HIF-1α, including NFκB1, which is involved in regulation of inflammation and cancer (Pucułek *et al*., 2018) .

The worldwide infection ratio of CagA positive and CagA negative strains is about 6:4, except for the East Asia region, where most strains are CagA positive. It has been reported that individuals infected with CagA positive strains of *H. pylori* are at a higher risk of peptic ulcer or gastric cancer than those infected with CagA negative strains (Palli *et al*., 2007).

2-8-2-Duodenal ulcer

The review evidence supports a strong causal relation between *H. pylori* infection and duodenal ulcer, as patients are more likely to be infected by virulent strains which later cause duodenal ulceration. Thus, eradication of *H. pylori* infection decreases the incidence of duodenal ulcers, and prevents its recurrence by reducing both basal gastrin release and acid secretion without affecting parietal cell sensitivity (Ahmed & Belayneh, 2019) . Perforated duodenal ulcer (PDU) is one of the most common surgical emergencies in this region and is associated with a substantial mortality rate of 6–30% (Low *et al*., 2008).

Among different investigated virulence factors, duodenal ulcer promoting gene A (dupA) has been found to be associated with duodenal ulcer (DU), but its effect was different in various geographical regions (Fatahi *et al*., 2019). *H. pylori* can elevate acid secretion in people who develop duodenal ulcers or hyper secretion of gastric acid can by itself evoke duodenal ulcers (Ahmed & Belayneh, 2019) .

The bacterium attaches to epithelial cells of the stomach and duodenum, then it causes damage to the cells by secreting degradative enzymes (urease, lipases, and proteases) and bacterial virulence factors (cytotoxin-associated gene protein (CagA) and vacuolating cytotoxin

(VacA)), and initiating a self-destructive immune response (Cover & Blaser, 2009).

2-8-3-Chronic Active gastritis

H. pylori infection is causally related to the onset of chronic active gastritis (Zhang *et al*., 2017). *H. pylori* is the most important etiological agent of chronic active gastritis and peptic ulcer disease. *H. pylori* infection is also epidemiologically related to gastric carcinoma and has been implicated in the pathogenesis of primary gastric marginal zone lymphomas (also known as lymphomas of the mucosa-associated lymphoid tissue).The pathogenic mechanisms leading from chronic active inflammation of the gastric mucosa to the development of the epithelial and lymphoid alterations that may result in ulceration, metaplasia, cancer, and lymphoma remain poorly understood. Progress in our understanding of these areas has been hampered in part by the lack of a well-characterized animal model. Several animal models have been developed to help understand the pathogenesis of *H. pylori* infection (Ikeno *et al*., 1999).

In an effort to develop a model that parallels as closely as possible the course of infection as it occurs in the human host (Takahashi *et al*., 1998).

(Ikeno *et al*., 1999) inoculated a human strain of *H. pylori* into the stomach of a *Mongolian jerboa*. After a short period, most of the *Mongolian jerboas* infected with *H. pylori* developed chronic active gastritis. Subsequently, intestinal metaplasia and chronic gastric ulcers developed in some infected *Mongolian jerboas* .

2-9-Treatment:

Currently, the main challenge in the field of *H. pylori* infection is antibiotic resistance, which influences the efficacy of eradication regimens. The latest systematic review and meta-analysis reported that the primary and secondary resistance rates to clarithromycin, metronidazole, and levofloxacin exceeded 15% (alarming levels) in all the World Health Organization (WHO) regions (Savoldi *et al*., 2018) .The traditional proton-pump inhibitor (PPI)-based triple therapy (PPI plus two antibiotics) has been used for eradicating *H. pylori* for more than 20 years. However, PPI-based triple therapy provides low treatment success (intention-to-treat [ITT] analysis below 80% in most studies) (Graham & Fischbach, 2010).

In 2012, the Maastricht IV/Florence Consensus Report recommended that PPI-clarithromycin-containing triple therapy should be abandoned in areas with clarithromycin resistance rates above 15% to 20%. In addition, bismuth-containing quadruple therapy (BQT) is recommended as a first-line treatment for eradicating *H. pylori* in areas with high or low clarithromycin resistance because of its high efficiency, safety, and tolerance. More, a number of studies were conducted to evaluate the efficiency of other regimens (eg, sequential, concomitant, hybrid therapy, high-dose PPI-amoxicillin dual therapy, vonoprazan [VPZ]-based triple therapy, probiotics supplemented triple therapy or combined with BQT) in *H. pylori* eradication. the review by (Malfertheiner *et al*., 2012).

Summarize the recent progress in *H*. pylori eradication .However, bacterial antibiotic resistance is still challenging the outcome of *H. pylori* eradication treatment. The "key" antibiotics in the treatment of *H. pylori* infection are clarithromycin and levofloxacin, and the prevalence of *H. pylori* strains resistant to these antibiotics has been increasing over the last decades (Megraud *et al*., 2013) .

3.1.Materials and Methods

3.1.1.Equipment and Instruments

The equipment and instruments used in this study are listed in Table (3-1)

Table (3-1) : Equipment and instruments

No	Equipment and instruments	Source
1.	Auto vortex	Bioneer , Korea
2.	Autoclave	Haramaya . Japan
3.	Automatic mono and multichannel pipette	Slamed . Germany
4.	Candle jar	Germany
5.	Deep freezer	Stuart Scientific . UK
6.	Digital camera	Sony , Japan
7.	Electrophoresis	Cleaver , scientific
8.	ELISA system	Biotech .USA
9.	Eppendorf centrifuge	Hitich , Germany
10.	Eppendorf tubes	Germany
11.	Hood	Fisher, scientific
12.	Incubator	Memmert . Germany
13.	Light microscope	Olympus . Japan
14.	Oven	Memmert .Germany
15.	Petri dish	Sterilin , England

16.	Plain tubes	DMD-DIDPO , Syria
17.	Refrigerator	Japan
18.	Sensitive balance	Sartorius . Germany
19.	Water distillater	GFL. Germany
20.	Water path	Memmert .Germany

3.1.2. Culture media, kits and reagents

Culture media, kits and reagents that used in this study are listed in Table (3-2)

Table (3-2) Culture media, kits and reagents

No	Culture media	Source
1	Blood agar base	Madrid , Spain
2	Brain –heart infusion broth	Madrid , Spain
3	Brain –heart infusion agar	Madrid , Spain
4	Muller – Hinton agar	Madrid , Spain
5	Nutrient broth	Himedia , India
6	Nutrient agar	Madrid , Spain
7	Skirrow media	Madrid , Spain
8	Urea agar base	Oxoid , USA
9	Urea supplement	Oxoid , USA

No	kits and reagents	Source
1	Ethanol 90%	Iraq
2	Glycerol	Fluka . Austria
3	*H. pylori* antibody rapid test kit	ACRO , USA
4	*H. pylori* antigen rapid test kit	ACRO , USA
5	Human IL-10(Interleukin 10) ELISA Kit	Elabscience , USA
6	Human IL-22(Interleukin 22) ELISA Kit	Elabscience, USA
7	Human Immunoglobulin A ELISA Kit	Bioassay technology, china
8	Human Immunoglobulin E ELISA Kit	Bioassay technology, china
9	Hydrochloric acid	Fluka , Austria
10	Normal saline	Iraq
11	Tris-buffer solution	Bioner , Korea

3.1.3. Antibiotics

All antibiotics used in this study are listed in Table (3-3).

Table (3-3) Types of antibiotic

Group	Purpose	Name	Concentration	Breakpoint
1	Antibiotic susceptibility test	Amoxillin (AX)	10 µg	**≥ 21<16**
		Clarithromycin (CLR)	15 µg	**≥ 23<21**
		Ciprofloxacin (CIP)	30 µg	**≥ 25<20**
		Doxycycline (DO)	30 µg	**≥ 19<17**
		Levofloxacin (LEV)	5 µg	**≥ 19<17**
		Metronidazole (MET)	5 µg	**≥ 24<17**
		Rifampicin (RA)	5 µg	**≥ 22<17**
2	Antibiotic for selective media	Polymyxin B	2.5 µg	
		Trimethoprim	5 µg	
		Vancomycin	10 µg	

3.2 .Methods

3.2.1. Gram stain

It consists of four solutions which include : crystal violet , iodine , ethanol , and safranin .

3.2.2. Catalase reagent

The reagent was prepared in 3% concentration by adding 3% of H_2O_2 to 100 ml of D.W. then stored in a dark bottle , it was used to recognize bacterial capability to produce catalase enzyme (Forbes *et al*., 2007) .

3.2.3. Oxidase reagent

The reagent was prepared immediately by dissolving 0.1g of tetra methyl-p-phenyl diamindihydrochloride in 10 ml of distilled water and saved in a dark bottle and cold place (Forbes *et al*., 2007) .

3.2.4. Normal saline solution

It was prepared by dissolving 0.85g of sodium chloride in a 90 ml of distilled water and sterilized by autoclave (Forbes *et al*., 2007).

3.2.5. Tris buffer solution

It was prepared by dissolving 1.21g of tris base in few amount of distilled water and then complete the volume to 1000 ml with distilled water (Forbes *et al*., 2007).

3.2.6. Phosphate saline solution

It was prepared by dissolving one tablet of PBS in 100 ml of D.W. then sterilized by autoclave 121 C° for 20 min, and stored at 4 C° until be used (Prasai *et al*., 2007).

3.2.7. Formalin solution (10%)

This solution was prepared at 10% concentration by adding 10 ml formaldehyde to 90% normal saline , the solustion was used to preservation and fixation of fresh tissues (Mohan & Pittman Jr, 2007).

3.3. Preparation of culture media

Preparation of culture media both culture media (solid and liquid) were prepared according to the installed manufacturer's instructions on the can. These media are:

3.3.1. Modified blood agar base (selective media)

Forty five (45) gram of blood agar base media were taken and suspended in one litter of D.W and sterilized in autoclave (121C° for 20 min) , and after that left to cool to 45 C° and the following antibiotics were added to it.

Amphotericin B 2.5 mg/ ml

Trimethoprim 5 mg/ml

Vancomycin 10 mg/ml

And 10% of human blood was added , mixed well then poured in petri dish (25 ml/one petri dish), leaving the media to cool and then saved in refrigerator for its use .

3.3.2 Skirrow media

Forty five (45) gram of blood agar base media were taken and suspended in one litter of D.W and sterilized in autoclave (121C° for 20 min)

, and after that left to cool to 45 C° and the following antibiotics were added to it.

This media consist of the following :

- Brain heart infusion agar
- Human blood (5%)
- Polymixin B 2.5 mg/ ml
- Trimethoprim 5 mg/ml
- Vancomycin 10 mg/ml

3.3.3. Urea agar base

It was prepared by dissolving 6 gm of urea agar base in 100 ml of D.W , sterilized by autoclave (121C° for 20 min) , left to cool and one vial of urea supplement (5 ml) was added to it, mix well and poured in sterile plain tube (5 ml/ tube) and saved in refrigerator until its used. This media was used to test the ability of bacteria to produce urease enzyme (MacFaddin, 2000).

3.3. 4. Brain heart infusion broth

This media was prepared by dissolving 3.7 gm of brain heart infusion broth in 100 ml of D.W, sterilized by autoclave (121C° for 20 min) , left to cool, mix well and poured in sterile plain tube (5 ml/ tube) and saved in refrigerator until its used. This media was used to activation of bacteria as well as preserved of biopsy through taken in endoscopic unit for culturing in microbiology lab (Atlas, 2010).

3.3. 5. Preserved media

This media was prepared in the same way of brain heart infusion broth except adding of glycerol (15-20%) and then poured in sterile plain tube (5

ml/ tube) and saved in refrigerator until its used. This media was used to preserved of bacteria for long time (Atlas, 2010).

3.3.6:Muller-Hinton Agar

Muller-Hinton agar medium was ready for conferring to the manufacturing company and it was used in antimicrobial susceptibility testing (Hendriksen, 2002).

3.4. Collection of samples and processing

This study involved collecting three different samples (blood, stool, and biopsy) from people who were suffering from abdominal pain and digestive problems. (121) blood samples were collected from different people. They were collected from the endoscopy unit of the Medical City of the Teaching Hospital of Hepatology and Gastroenterology and the General Hospital of Essaouira with the help and supervision of the doctor and specialized staff and after the approval of the patients for the period from September 2020 to April 2021 as shown in Figure (3.1).

3.4.1.- Blood samples

Three (3) ml of blood samples was collected from each patient and control , put in gel tube and preserved in safety cool pox , transported through two hours to microbiology lab, centrifugation and then divided to two parts , first part for rapid *H. pylori* diagnosis test and second part was used to determination of, immunological parameter interleukin10,interleukin 22,IgA,IgE, by ELISA methods.

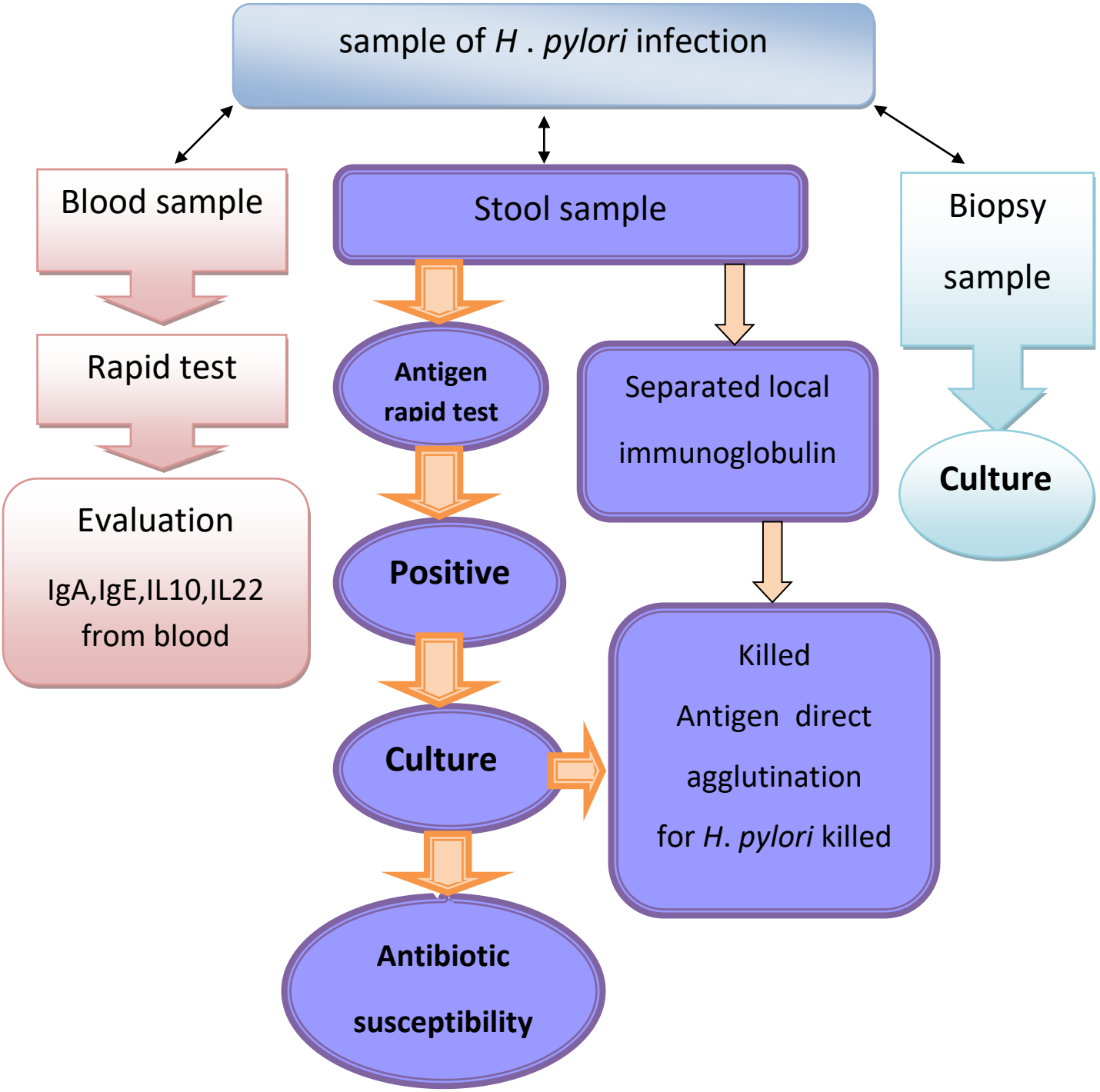


Figure (3.1) : Study Design

3-4-2-Antibody rapid test

The principle of serum antibody rapid test of *H. pylori* was based on qualitative membrane immunoassay for the detection of *H. pylori* antibodies in whole blood, serum, or plasma . In this test, anti-human IgG is immobilized in the test line region of the test. After sample is added to the sample well of the device, it reacts with *H. pylori* antigen coated particles in the test. This mixture migrates chromatographically along the length of the test and interacts with the immobilized anti-human IgG. If the specimen contains *H. pylori* antibodies, a colored line will appear in the test line region indicating a . The test is used to diagnose the bacteria in blood samples . In the absence of bacterial infection, only the red coloration appears on the letter C (control line), while in the case of infection in addition to the red coloration of the package control line, a red balloon band will appear on the letter T (result line) as shown in the figure (3.2).

Figure (3.2) : Antibody rapid test

In the absence of bacterial infection, only the red coloration appears on the letter C (control line), while in the case of infection in addition to the red coloration of the package control line, a red balloon band will appear on the letter T (result line)

3.4.3. Stool samples

1. Stool antigen rapid test

The test is used to diagnose the bacteria in stool samples . small samples of stool specimens collected from three different parts of the stool sample wire transferred to s vial with diluents, vigorously agitated and after two minutes of resting the tuba, dropping sound two to three drops into the round window of the test cassette. Riding was made after 10 minutes of incubation at room temperature, and based on the appearance of colored lines across the central window of the cassette, two lines, C (control) and T (test), indicted positive test, only one line in C indicted negative result. A pale colored line in T was also considered positive (Silva *et al.*, 2010).as it is shown in Figure (3-3).

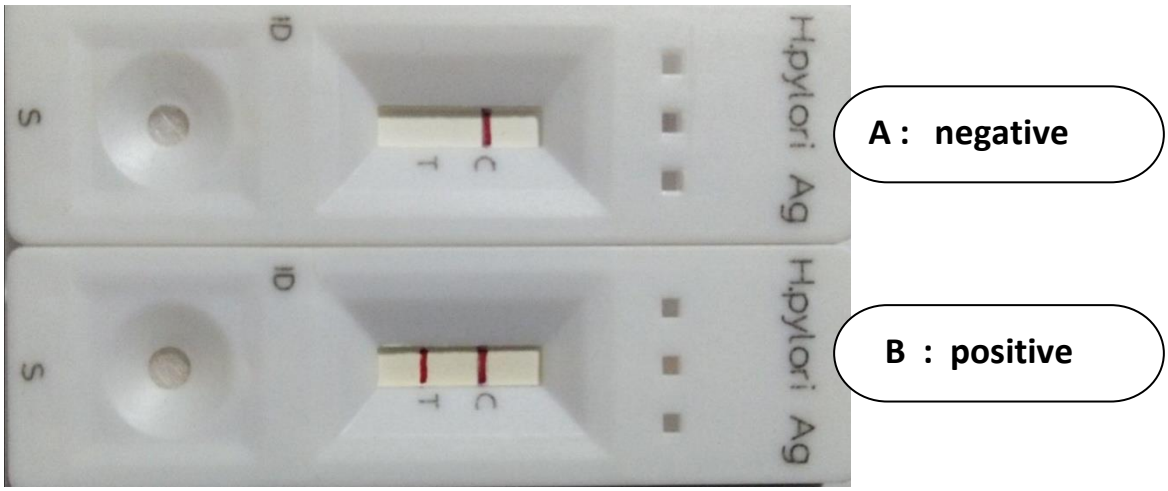


Figure (3-3): Stool antigen rapid test

appearance of colored lines across the central window of the cassette, two lines, C (control) and T (test), indicted positive test, only one line in C indicted negative result

Stool antigen tests (SATs) are noninvasive diagnostic modules for *H. pylori* infection and were introduced after the urea breath test (UBT). Early SATs used immunochromatography (ICA) based on polyclonal antibodies as in the following steps:

1-A Proximally 0.25 gram of stool was transported by a wooden stick into a tube containing the buffer solution to prepare a suspension which is used to mitigate the sample .

2- The sample was mixed well before dispensed in card .

3-The outer cover of the card was removed upward before the direct use.

4-A two drops of suspension solution added to the circular region of the card.

5-The result was read within 15 minutes.

2.Bacterial isolation

A small sample of stool was collected in sterile cub from each patient and control and preserved in safety cool pox , transported through two hours to microbiology lab, suspended with normal saline solution and then inoculation of brain heart infusion broth as activation media for 24 hr under microaerophilic condition , after this transported to selective media (Skirrow agar and modified blood agar) , incubation at 37C° in microaerophilic condition for 3-5 days.

3.4.4. Biopsy samples

Small pieces of gastric biopsy was collected directly in endoscopic unite in tube which containing brain heart infusion broth respectively . Tubes were preserved in cool pox and transported to microbiology lab within two hours was used to activation and cultivation of bacteria.

3.5. Culturing of *H. pylori*

The antral biopsy specimens were transported to the microbiology laboratory immediately within less than two hour in 2-2.5 mL brain heart infusion broth as a transport medium. Incubated at 37 C° for 24 hours then subcultured on modified blood agar base and skirrow agar for 3-5 days.

3.6. Identification of *H. pylori*

3.6.1.Morphological identification of *H. pylori*

This is depending on the morphological properties of colonies such as color ,size of colony ,shape, condition of growth ,edge and elevation of texture part , also microscopic examination as Gram staining ,shape and size of bacterial cell ,and arrangement of these cell.

3.6.2.Biochemical diagnostic test of *H. pylori*

3.6.2.1.Gram stain

Smear was taken from bacteria and Gram stain was applied on the smear

.

3.6.2.2.Oxidase test

A small drop of reagent place on filter paper and then small portion of bacterial colonies was spread on this paper by wooden stick , positive result appeared as color change to purple , while negative result appeared as no color change . This test depend on the presence of certain bacterial oxidase that would catalyze the transport of electrons between electron donors in bacteria and a redox dye (Tetramethyl-p-phenylene- diamine dihydrochloride) (Forbes *et al*., 2007).

3.6.2.3.Catalase test

The isolates were tested for catalase activity by using slide method in which the sterile wire loop is stacked on the surface of the pure colony and transferred to a microscope slide and one or two drop of 3% H2O2 were added onto the organism on the slide and observed for immediate oxygen bubble formation (Forbes *et al*., 2007) .

3.6.2.4.Urease Test

The urease test was applied according to (MacFaddin, 2000). The pure isolates were inoculated heavily on the entire surface of urea agar and stab with loop wire. The tubes were inoculated at 37°C in the incubator. The formation of purple color was examined after 4 hr.

3.6.3.Diagnosis of *H .pylori* by agglutination test

3.6.3.1.Separation method for secretary immunoglobulin from feces by PEG (Al-Saadi *et al*., 1998).

1-Mixing and homogenize the stool sample inside the collection vessel with a sterile glass rod With a length of (15) mm and a diameter of (0.3) mm

2- About (2) grams of homogeneous feces were taken and placed in a sterile glass beaker with a volume of 10 ml, and 10 ml of formalin salt solution (0.5%) was added, and the stool was mixed and homogenized with this solution with a stir bar sterile vitreous

3- The fecal solution was placed in a centrifuge tube and placed in a centrifuge at 3500 cycles for a duration of half an hour

4- The feces were collected and discarded again at 4000 rpm for 10 minutes

5- The suds were collected, the sediment was left and (5) ml was taken. From the sediment and an equal volume of PEG was added to it at a concentration (6%) and then left at room temperature for half an hour

6- The solution was centrifuged for half an hour at a speed of 3500 rpm

7- The sediment was left and the precipitate was taken and then dissolved in 1 ml of The functional salt solution is then stored in the refrigerator at 4°C

3.6.3.2.Preparation of heat killed bacterial antigens *H. pylori*

Heat killed antigen was prepared from many of bacterial isolated in this study in added to bacteriologically and molecularly isolates were obtained from the microbiology laboratories - University of Baghdad - College of Science, Department of Life Sciences, to confirm the bacterial diagnosis.

The isolates were cultured at 37˚C overnight on nutrient agar by spreading method.

- The bacterial growth was harvested by adding sterile normal saline in test tube, and then heat-killed (30min at 60 ˚C), centrifuged at 2500 rpm for 10 min.
- The supernatant was avoided; the precipitate was washed three times with normal saline and suspended in normal saline at concentration of 1×10^9 cell/ml then tested by culturing on blood agar.
- The sterile examination was performed on the antigenic suspected by taking specimen from suspected by loop and streaking it on solid media and then incubated at a degree of 37 ˚Ċ for 24 hr. in the event that bacterial growth does not appear, the prepared antigen is ready for use

Killed antigens suspension stored in refrigerator - 20 ˚C for use (Agren *et al.*, 1998).It used to detected specific antibody.

3.6.4.Agglutination test

This is done to ensure the specificity of separating Ab from the stool of patients using the Ag bacterial preparation.

1.Placed one drop of heat killed bacterial antigen on disposable card or a ringed glass slide.

2. 30-50 µl of previously extracted immunoglobulin was added.

3. Rotated by the hand for 2 min.

4. Examined the agglutination reaction under a bright light.

5.Reading the result, if the suspension remains homogenous and slight milky in appearance(negative reaction), either agglutination of the particles and slight clearing of the suspension occurs (positive reaction).

3.6.5. Antimicrobial susceptibility of *H. pylori*

In this test, the antibiotics names and its standard inhibition diameters were used as it recommended by (Schmidt-Malan et al., 2016)

1. The inoculums used in this test were prepared by adding (3-5) isolated colonies grown on modified blood agar plate to 5 ml of sterile normal saline and compared with (1.5X 10 cell/ml) McFarland standard tube.

2. A sterile swab was used to acquire inoculums from the bacterial suspension. These inoculums were streaked on a Mueller-Hinton agar (MHA) plate and left to dry.

3. The antibiotics disks were placed on the surface of the medium at consistently spaced intervals with flamed forceps or a disc applicator and incubated for 24 hr. at 37 °C.

4. Inhibition zones were measured by using a ruler or a caliper and compared with the zones of inhibition determined by the Clinical Laboratory Standards Institute to conclude the resistance or sensitivity of the organism to each antibiotic.

5- The measurement obtained from the individual antibiotics was compare with the standard table to determine the sensitivity zone.

3-7-Human IL-10(Interleukin 10) ELISA Kit:

3-7-1-Test principle:

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IL-10. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-10 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human IL-10, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human IL-10. You can calculate the concentration of Human

IL-10 in the samples by comparing the OD of the samples to the standard curve as it is shown in Figure (3-4).

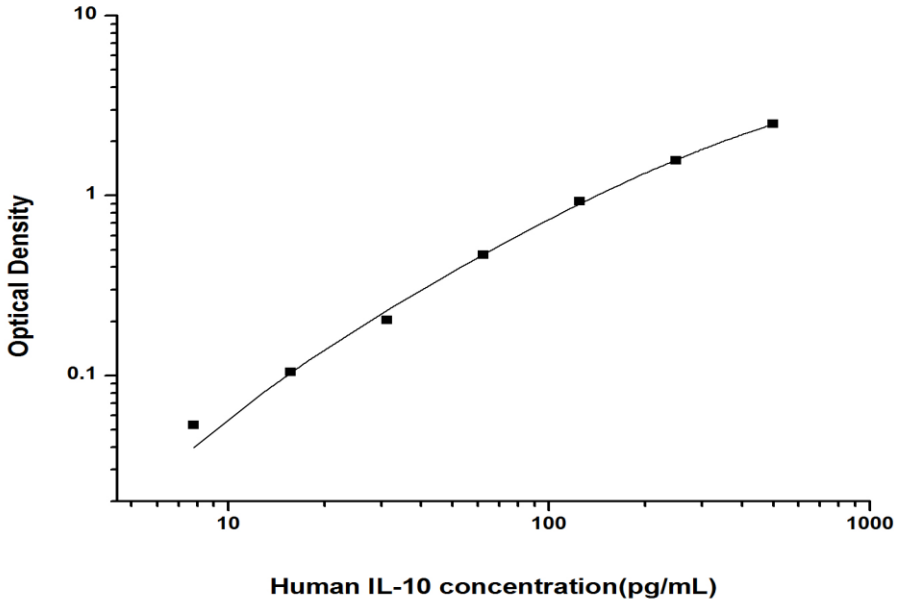


Figure (3-4): Standard curve of IL10

3-7-2- Reagent Preparation :

1. All reagents to room temperature (18~25℃) before use. Follow the micro plate reader manual for set-up and preheat it for 15 min before OD measurement.

2. **Wash Buffer:** Dilute 30 mL of concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Washing Buffer .Note: if crystals have formed in the concentrate, warm it in a 40 ° C water bath and mixed it gently until the crystals have completely dissolved.

3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Added 1.0 mL of Reference Standard &Sample Diluents, lilted stand

for 10 min and invert it gently several times. After it dissolves fully, mixed it thoroughly with a pipette. This reconstitution produces a working solution of 500 pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 0 pg/mL. Dilution method: Taked 7 EP tubes, added 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 500 pg/mL working solution to the first tube and mixed up to produce a 250 pg/mL working solution. Pipette 500uL of the solution from the former tube into the latter one according to these steps. The illustration below is for reference . Note: The last tube is considered empty. Do not suck the solution from the previous tube curve as it is shown in Figure (3-5).

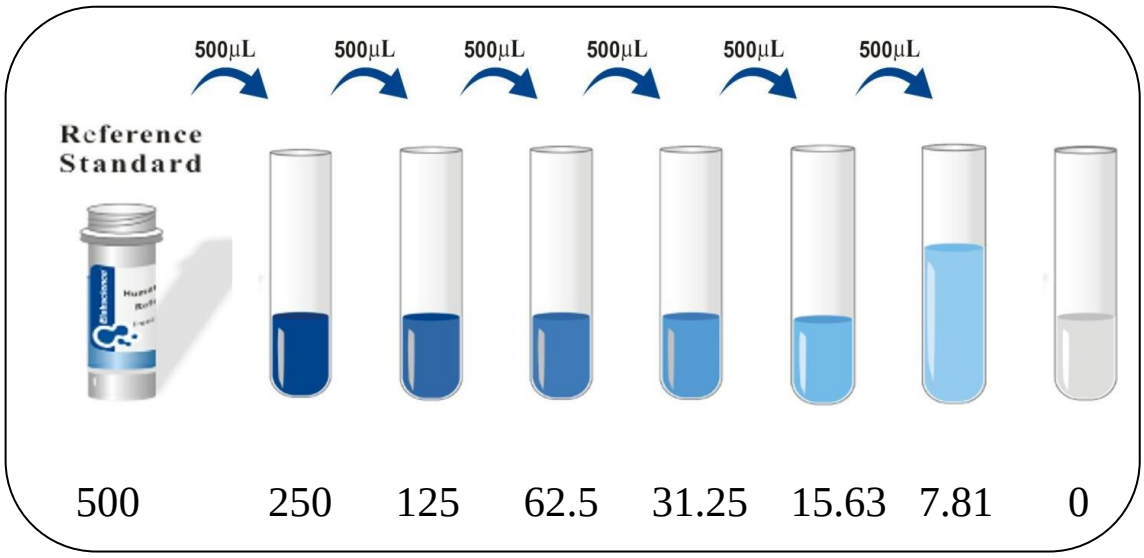


Figure (3-5): Standard working solution IL10

4. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (100 µL/well).In preparation, slightly more

than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent.

5. **Concentrated HRP Conjugate working solution:** Calculate the required amount before the experiment (100μL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100× Concentrated HRP Conjugate to 1× working solution with Concentrated HRP Conjugate Diluents.

3-7-3-Assay Procedure (Interleukin 10)

1. Prepared all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.

2. Determined the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.

3. Added 50μl standard to standard well. **Note**: Don't added antibody to standard well because the standard solution contains biotinylated antibody.

4. Added 40μl sample to sample wells and then add 10μl anti-IgA antibody to sample wells, then added 50μl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mixed well. Covered the plate with a sealer. Incubate 60 minutes at 37°C.

5. Removed the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirated all wells and wash 5 times with wash

buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Added 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. Added 50µl Stop Solution to each well, the blue color will change into yellow immediately.

8. Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minuets after adding the stop solution.

3-8-Human IL-22(Interleukin 22) ELISA Kit

3-8-1-Test principle:

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IL-22. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-22 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human IL-22, biotinylated detection antibody and Avid in-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The

OD value is proportional to the concentration of Human IL-22. You can calculate the concentration of Human IL-22 in the samples by comparing the OD of the samples to the standard curve as it is shown in Figure (3-6).

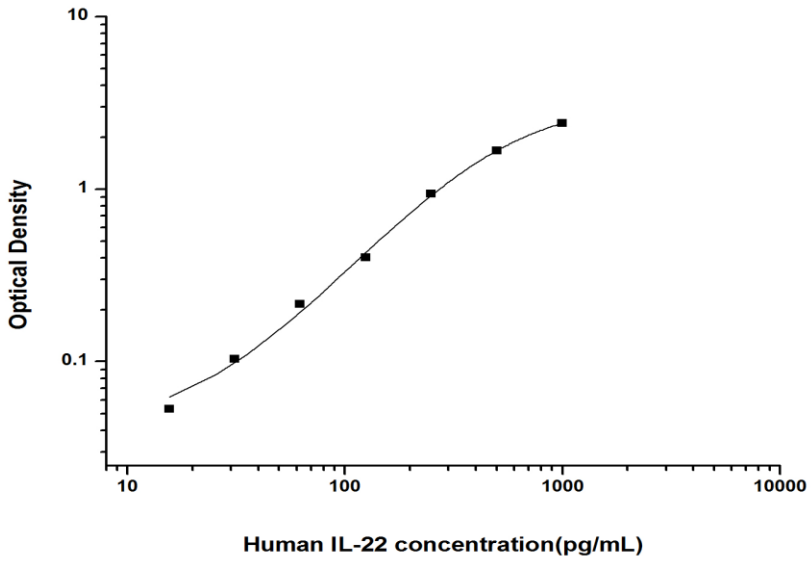


Figure (3-6): Standard curve of IL22

3-8-2-Reagent preparation:

1. Bringing all reagents to room temperature (18~25°C) before used. Followed the micro plate reader manual for set-up and preheat it for 15 min before OD measurement.
2. **Wash Buffer:**

   Dilute 30 mL of concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer .Note: if crystals have formed in the concentrate, warm it in a 40°C water bath and mixed it gently until the crystals have completely dissolved.

3. Standard working solution:

Centrifuge the standard at 10,000×g for 1 min. Added 1.0 mL of Reference Standard &Sample Diluent, letting it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 1000 pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 1000, 500, 250, 125, 62.5, 31.25, 15.63, 0 pg/mL. Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 1000 pg/mL working solution to the first tube and mix up to produce a 500 pg/mL working solution. Pipette 500uL of the solution from the former tube into the latter one according to these steps. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube as it is shown in Figure (3-7).

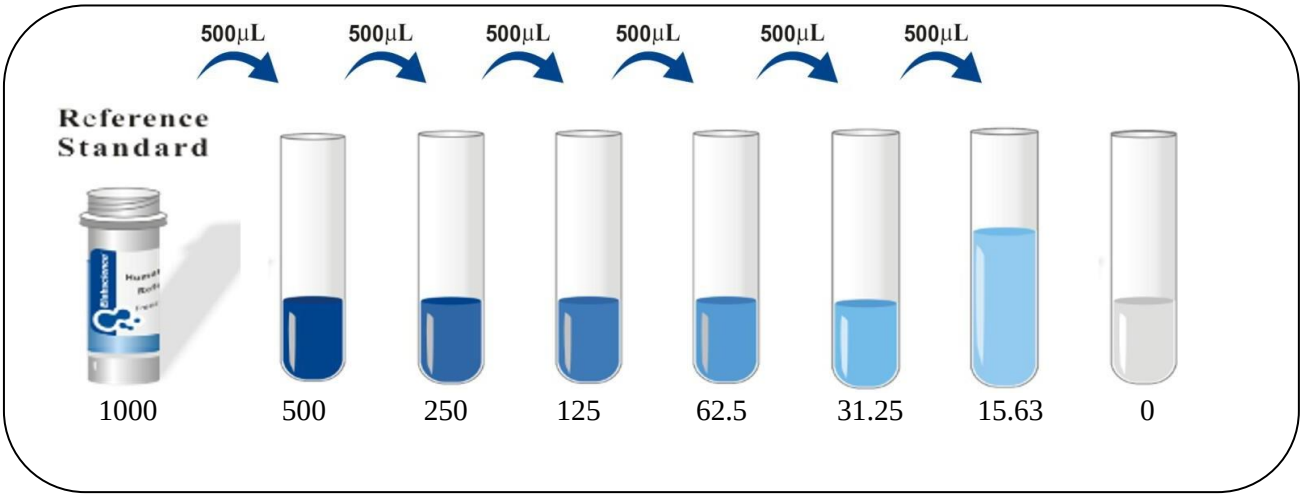


Figure (3-7): Standard working solution IL22

4. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent.
5. **Concentrated HRP Conjugate working solution:** Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100× Concentrated HRP Conjugate to 1× working solution with Concentrated HRP Conjugate Diluents.

3-9- Human Immunoglobulin A ELISA Kit :

3-9-1-Assay Principle:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human IgA antibody. IgA present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human IgA Antibody is added and binds to IgA in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IgA antibody. After incubation unbound.

3-9-2-Reagent Preparation :

All reagents should be brought to room temperature before use.

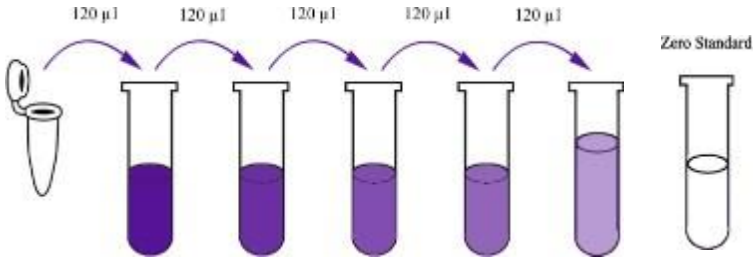
1-**Standard** Reconstitute the 120µl of the standard (640ng/ml) with 120µl of standard diluent to generate a 320ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (320ng/ml) 1:2 with standard diluent to produce 160ng/ml, 80ng/ml,

40ng/ml and 20ng/ml solutions. Standard diluent serves as the zero standard(0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows in Table (3-4).

2-Wash Buffer Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Table (3-4) Reagent Preparation

320ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
160ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
80ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
40ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
20ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
640ng/ml	320ng/ml	160ng/ml	80ng/ml	40ng/ml	20ng/ml

3-9-3-Assay Procedure : (IgA and IgE)

1. All reagents, standard solutions, and samples were prepared according to the instructions. The reagents were placed at room temperature before use. The examination is at room temperature.

2. Determining the number of strips required for the assay. Strips have been inserted into the frames for use. Storing unused strips at 2-8 ° C.

3. A 50 µl of the standard well was added to the standard well. Note: The antibody is not added to the standard well because the standard solution contains an antibiotic.

4. A 40 µl sample was added to the well sample and then 10 µl of IgA antibody was added to the well samples, then 50 µl of Streptavidin-HRP was added to the well and standard well samples (not a good blank control). Mixed it well. Covered the plate with sealant. Incubate 60 minutes at 37 ° C.

5. The sealant was removed and the panel was washed 5 times with washing solution. Soaking wells with at least 0.35ml of washing solution for 30 seconds to 1 minute for each wash. For automatic washing, all wells are washed and cleaned 5 times with laundry storage, and wells filled with laundry storage. Cleaning the bandage on a tissue or other absorbent material.

6. A 50 µl of substrate A solution was added to each well and then 50 µl of substrate B solution was added to each well. Incubate plate covered with fresh sealant for 10 minutes at 37 ° C in the dark.

7. Added 50 µl of suspension solution to each well, the blue color will turn yellow immediately.

8. Determining the optical density (OD value) of each well immediately using a micro plate reader set at 450 nm within 10 minutes after adding the stop solution

The micro-plate reader must be opened in advance at 450 nm for the user, the device is preheated, and the test parameters set, as shown in Figure (3-8).

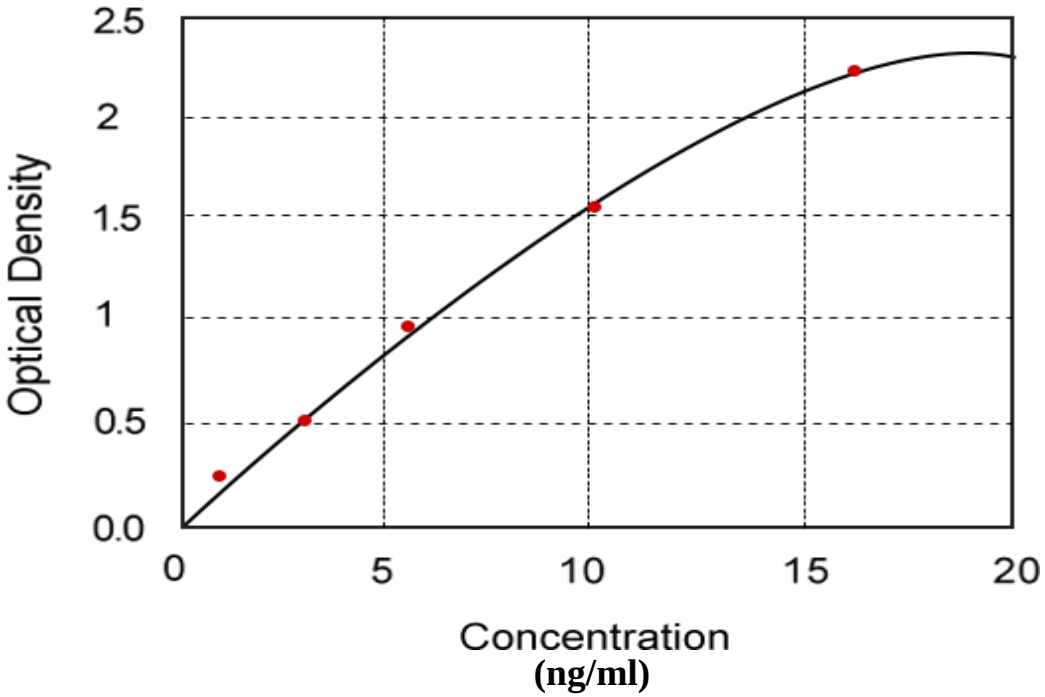


Figure (3-8): Standard curve of IgA .IgE

3.10: Statistical analysis

Data were processed and analyzed with one way independent sample using statistical program social science (SPSS version 23)and the results were expressed as (Mean ± S.D). P-values below 0.05 were considered to be statistically significant, in addition correlation with different parameters were done for cytokines and other studied parameters (Jacobson *et al*., 2011) .

chapter (2)

properties of Nucleons and Nuclei

2.1 The nucleus, its size and shape we begin this with a brief discussion of the size and shape of atomic nuclei

2.1.1 The nuclear radius:

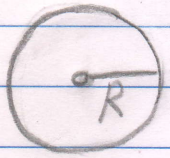
$$R = r_0 A^{1/3}$$ r_0 : radius constant

or called the nuclear unit radius

$$r_0 = 1.25\ fm = 1.25 * 10^{-13}\ cm$$

2.1.2 The density of nucleus

$$\rho_N = \frac{M_N}{V_N}$$



ρ_N : nucleus density

M_N : nucleus mass

V_N : nucleus volume

$$V_N = \frac{4}{3}\pi R^3$$

$$R = r_0 A^{1/3}$$

$$V_N = \frac{4}{3}\pi r_0^3 A$$

$$\rho_N = \frac{M_N}{V_N} = \frac{A\ a.m.u}{\frac{4}{3}\pi R^3}$$

$$= \frac{A}{\frac{4}{3}\pi r_0^3 A}$$

$$\rho_N = \frac{3}{4\pi r_0^3} = \rho_0 = \text{Constant}$$

2.2 Mechanical Effects Due to Orbiting and Spinning of Nucleons

2.2.1 Orbital angular momentum of nucleons

The angular momentum of a nucleon due to its orbiting motion can have only certain discrete values given by $\hbar[l(l+1)]^{1/2}$ where $\hbar$ is the unit of action equal $\frac{h}{2\pi}$ where h is the planck's constant and l is a positive integer including zero.

Thus $l = 0, 1, 2, 3, 4, 5, 6 \ldots\ldots, (n-1)$

$L = \hbar\sqrt{l(l+1)}$, the observable maximum value of the orbital angular momentum is $l\hbar$.

Table (2.1) values of l and their spectroscopic symbols.

Numerical	0	1	2	3	4	5	6	7
spectroscopic symbol	s	p	d	f	g	h	i	j

2.2.2 Spin angular momentum of nucleons

Besides orbiting around, each nucleon spins motion is also quantized. The only value of angular

momentum due to spin motion is given by: $\hbar[s(s+1)]^{1/2}$ where s is $\frac{1}{2}$ for protons, neutrons and electrons. The observable maximum value of the spin angular momentum is $s\hbar$

$$S = \hbar\sqrt{s(s+1)}$$

2.2.3 Total angular momentum of nucleon

The summation of the orbital and spin angular momentum of a nucleon is its total angular momentum j given by: $j = l \pm s$

The corresponding total angular momentum of the nucleon is given by $\hbar[j(j+1)]^{1/2}$ Max. total angular momentum is $j\hbar$

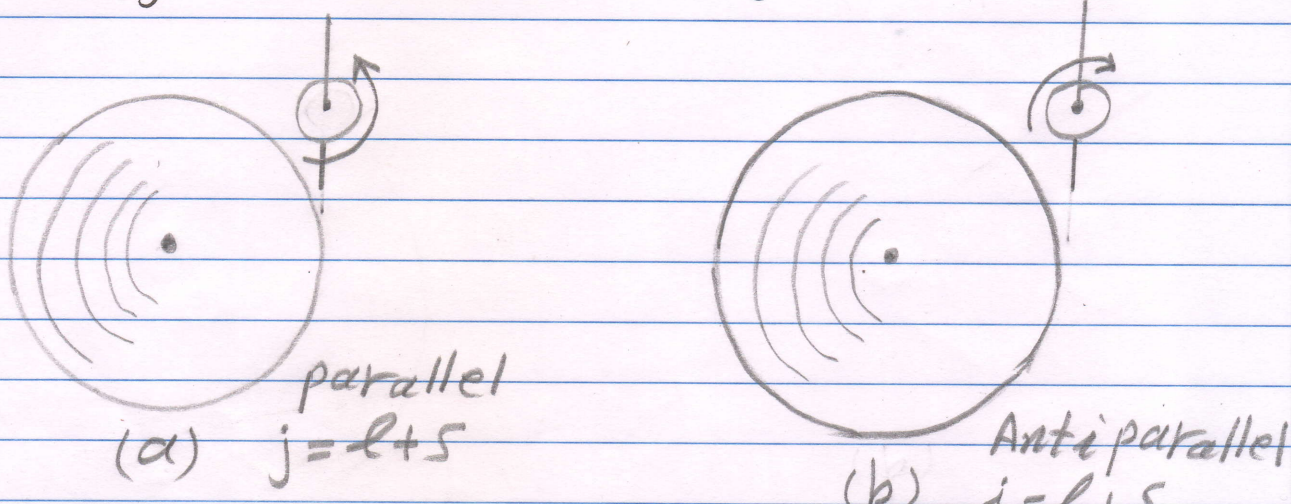


Fig. (2.1) The orientation of nuclear spin with respect to orbital motion

when $l=0 \Rightarrow j=0+\frac{1}{2} \Rightarrow j=\frac{1}{2}$ has a single value $j=\frac{1}{2}, \frac{3}{2}, \frac{5}{2}, \frac{7}{2}, \frac{9}{2}, \ldots$

when $l=0$, S, j has a single value of $+\frac{1}{2}$ as j can has only positive values. Thus the only values of j possible are positive half-integers:

2.3 Magnetic quantum numbers

To each of the above described angular momentum numbers (l, S and j) there exists a magnetic analogue (m_l, m_s and m_j)

a- Magnetic orbital quantum number (m_l)

Can take any one of $(2l+1)$ possible positive and negative integral values lying between $+l$ and $-l$ including Zero. If the orbital angular momentum quantum number l is function (= 3). m_l can have any one of the following seven ($=2l+1$) values: 3, 2, 1, 0, -1, -2, -3. Not that l can have only positive integer values including Zero.

(b) Magnetic spin quantum number m_s

Can have only one of two values $+s$ or $-s$ since for protons, neutrons and electrons, s has the only value of $\frac{1}{2}$, m_s can be only either $+\frac{1}{2}$ or $-\frac{1}{2}$

(c) Magnetic total quantum number m_j

Can take any one of $(2j+1)$ possible values Thus: $m_j = j, (j-1), \ldots \frac{1}{2}, -\frac{1}{2} \ldots -(j-1), -j$

2.4 Total angular momentum of the nucleus

When two or more nucleons are able to come togther to form a nucleus the components of motion of individual nucleons interact with one another which leads to a resultant total angular momentum of the nucleus as whole. This resultant determines the nuclear angular momentum is given by $\hbar[I(I+1)]^{1/2}$ where I is the corresponding total nuclear angular momentum quantum number. Max. value is $I\hbar$.

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قسم الكيمياء للدراسة الصباحية

((عنوان التجربة))

((تنقيه ملح الطعام NaCl))

الاسم/حسن صالح هادي جاسم
المرحلة/ الثانيه
المادة/ الكيمياء الاعضويه العملي
اسماء الشركاء/ حسن عامر محمد/حوراء علي عبد اللطيف/بنين مكي علاوي

تاريخ اجراء التجربة. 2021/11/4

Certification

I certify that this thesis was prepared under my supervision at the Department of Biology, College of Science, University of Babylon as partial fulfillment of the requirement for the degree of Master of Science in Biology – Microbiology.

Supervisor

Prof. Dr. Azhar Omran Althahab

College of Science

University of Babylon

/ /2021

In view of the available recommendation, I present this thesis for evaluation by the Examining Committee.

Assist Prof. Dr. Adi Jassim Abd AL-Rezzaq

Head of Biology Department

College of Science

University of Babylon

/ /2021

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