Chapter One

1. Introduction and Objective

1.1 Introduction

Helicobacter pylori is Gram-negative, microaerophilic spiral rod-shaped bacteria that lives just beneath the antral gastric mucous layer, on the surface of epithelial cells. Stomach infection with this organism causes inflammation of the gastric mucosa, which can lead to gastritis, duodenal or gastric ulcer and even in rare cases to gastric carcinoma or Mucosa Associated Lymphoid Tissue (MALT) lymphoma. Over 80% of individuals infected with the bacterium are asymptomatic (Covacci *et al.*, 1999).

Approximately, 50% of the world's population is believed to be infected with *H. pylori*. Most infections are probably acquired in childhood (Czesnikiewicz-Guzik *et al*., 2004), but geographic area, age, race, socioeconomic status, and hygiene seem to play roles in the frequency of *H .pylori*. Most of the information about *H. pylori* infection rates comes from sero-prevalence studies. Higher rates of infection tend to occur at a younger age in developing countries compared to developed countries and in regions characterized by lower socioeconomic status and higher density living. Looking at racial differences in the US, it is found that whites of non-Hispanic origin have lower prevalence of infection compared to African-Americans or Hispanics (Brown, 2000).

It has been speculated that dental plaque might harbor *H. pylori* and, therefore, might be a source of gastric infection (Czesnikiewicz-Guzik *et al*., 2004).

Helicobacter pylori infection is linked to conditions of the upper gastrointestinal tract, including peptic ulcer, gastric adenocarcinoma, and MALT lymphoma. It has also been associated with wide variety of extra gastric and non-gastrointestinal

conditions. However, the evidence in support of *H. pylori* infection as a cause of the non-gastrointestinal tract diseases is not widely understood (Bettina and Alexander., 1999).

Bettina and Alexander (1999) reviewed the medical literature in regard to putative association of *H. pylori* infection and non-gastrointestinal tract condition, notably cardiovascular, autoimmune, and dermatological diseases.

Helicobacter pylori has also been recognized as a risk factor for the development of both gastric carcinoma and mucosal-associated lymphoid tumor. Although not widely known, *H. pylori* can also affect organ systems outside of the gastrointestinal tract. It is now apparent that *H. pylor*i can infect the skin, liver and heart and these infections may produce a number of different disease states (Utas *et al*., 1999).

Helicobacter pylori (*H.pylori*) is recognized as the main etiological agent of gastritis in human and also an essential factor in the pathogenesis of peptic ulcer (Megraud *et al* .,1989).There is evidence that the microorganism is also involved in the pathogenesis of gastric carcinoma and MALT type gastric lymphoma(Parsonnet *et al.*,1991).The microorganism is also responsible for one of the most frequent chronic bacterial infections involving more than 50% of the world population (Graham *et al.*, 1991).

In developed countries,children and adolescents are only infrequently infected and in adults over 50 years of age the seroprevalance of infection ranges from 30% to 60% . In United States , serologic evidence of *H.pylori* is rarely found before age 10 but increase to 10% in those between 18 and 30years of age and 50% in those >60 years . On the other hand in developing countries up to one half of 10 years old children are infected with *H.pylori*. In Nigeria , the infection involves up to 92% of all children elder than 10 years (Graham *et al* .,1991) . High rates of infection are also observed in Chile and other developing countries .

Infection with *H. pylori* was diagnosed by biopsy based tests (urease test,direct stain ,culture ,and histology) or non biopsy based tests (serology and urea breath test) serological tests are non –invasive and were used to detect immunoglobulin G (IgG) antibodies in sera of *H. pylori* infected subjects . The enzyme-linked immuno assay (ELISA) test is very widely used in epidemiological and post treatment studies .

1.2 Objectives of the study

1.2.1 General Objective:

The aim of this study was to detect antibodies raised against *H.pylori* in asymptomatic healthy people in Saudi Arabia.

1.2.2.Specific Objective:

1. Comparised between ELISA, IIFT, Western blot Technique to Diagnosis Helicobacter pylori .

2.To determine the risk factors age, gender in the development of the disease .

3.To use ELISA, Stool antigen, western blot, and IIFT Euoline tests as techniques to be used for identification of these pathogenic organisms in comparison with other techniques .

4.To assess the sensitivity and specificity of the above mentioned 3- tests for diagnosis of *H.pylori* infection .

1.3 Rationale

Helicobacter pylori infection occurs throughout the world and causes gastrodudenal diseases in all groups, it is wide spread in Saudi Arabia but few large studies exist on the epidemiology of *H.pylori* infection in Saudi Arabia (ALMoagel *et al* 1990)

Helicobacter pylori is considered to be the etiologic agent of chronic gastritis type B . The Fact that bacteria exist in the stomach mucous membranes has been known for about 100 years . The association with gastritis and stomach ulcers was first suspected in the forties . The rate of infection increases with the age (Borody *et al.*,1989).

It is prevalent world wide about 50% of all people are infected and it has been found in epidemiological studies that the rate of infection increases with the age.

Antibodies against *H.pylori* occur in about 70% of patients with chronic active gastritis and are associated with ulcer conditions in 60% to 90% of all cases .

The large majority of infections remains clinically asymptomatic and the late consequences are MALT lymphomas and adenocarcinomas . The infectious agent persistence promotes relapses through recolonization by residual agents.

A complete and permanent eradication of the bacteria in diagnosed *H.pylori* infection in children , adolescents and adults lead to a reduction of the relapsing rate of 80% in case of peptic ulcers and 20% in duodenal ulcers.

The serological tests are less expensive and less stressful for the patients ,especially for children (Graham, *et al.*,1991).

Chapter Two

2. Literature Review

2.1 History

Helicobacter pylori was first discovered in the stomachs of patients with gastritis and stomach ulcers in 1982 by Barry Marshall and Robin Warren in Perth, Western Australia (Borody *et al*., 1989).

At that time, the conventional thinking was that; no bacterium can live in the human stomach, as the stomach produced extensive amounts of acid. Marshall and Warren rewrote the textbooks with reference to what causes gastritis and gastric ulcers. Marshall drank a beaker of *H. pylori* culture. He became ill with nausea and vomiting several days later. An endoscopy ten days after inoculation revealed signs of gastritis and the presence *of H. pylori.* These results suggested *H. pylori* was the causative agent of gastritis. Marshall and Warren went on to demonstrate that antibiotics are effective in the treatment of many cases of gastritis. In 1987, the Sydney gastroenterologist Thomas Borody invented the first triple therapy for the treatment of duodenal ulcers (Borody *et al.*, 1989).

The bacterium was initially named *Campylobacter pyloridis*, then renamed *C. pylori* (*pylori* = genitive of pylorus) to correct a Latin grammar error. When 16S rRNA gene sequencing and other research showed in 1989 that the bacterium did not belong in the genus *Campylobacter*, it was placed in its own genus, *Helicobacter*. The genus derived from the ancient Greek *hĕlix*/ἕλιξ "spiral" or "coil". The specific epithet *pylōri* means "of the pylorus" or pyloric valve (the circular opening leading from the stomach into the duodenum), from the Ancient Greek word *πυλωρός*, which means gatekeeper (Sebestian and Pierre.,2002).

2.2 Classification

The Kingdom is bacteria, the phylum is proteobacteria.The class is Epsilonprotbacteria, Order is *Campylobacter* ales, belong tofamily Helicobacteraceae, Genus is *Helicobacter*, and specie is *pylori*.

Table 1: Characteristics of selected *Helicobacter* species (Kusters *et al*., 2006)

Species	Primary mammalian host	Pathology	Animal model
Gastric *Helicobacter* spp.			
H. pylori	Human, primate	Gastritis, peptic ulcer disease, gastric adenocarcinoma, MALT lymphoma	Mouse, Mongolian gerbil, guinea pig, gnotobiotic piglet
H. felis	Cat, dog, mouse	Gastritis in natural host; may cause peptic ulcers or gastric adenocarcinoma in mouse	Mouse
H. mustelae	Ferret	Gastritis, peptic ulcer disease, gastric adenocarcinoma, MALT lymphoma	None
H. acinonychis	Cheetah, tiger, other big cats	Gastritis, peptic ulcer disease	Mouse
H. heilmannii	Human, dog, cat, monkey, cheetah, rat	Gastritis, dyspeptic symptoms, MALT lymphoma	Mouse
Enterohepatic *Helicobacter* spp.			
H.hepaticus	Mouse, other rodents	Proliferative typhlocolitis, hepatitis, hepatocellular carcinoma	None

2.3 Bacteriology

Helicobacter pylori is a helix-shaped (classified as a curved rod, not spirochaete) Gram-negative bacterium, about 3 micrometres long with a diameter of about 0.5 micrometre. It is microaerophilic;that is,it requires oxygen,but at lower concentration than is found in the atmosphere. It contains a hydrogenase which can be used to obtain energy by oxidizing molecular hydrogen (H_2) that is produced by intestinal bacteria (Olson and Maier, 2002).

It produces oxidase, catalase, and urease. It is capable of forming biofilms and can convert from spiral to a possibly viable but nonculturable coccoid form both likely to favor its survival and be factors in the epidemiology of the bacterium. The coccoid form can adhere to gastric epithelial cells in vitro (Liu *et al*., 2006).

Helicobacter pylori possesses five major outer membrane protein (OMP) families. The largest family includes known and putative adhesions. The other four families include porins, iron transporters, flagellum-associated proteins and proteins of unknown function. Like other typical Gram-negative bacteria, the outer membrane of *Helicobacter pylori* consists of phospholipids and lipopolysaccharide (LPS) (Kusters *et al*., 2006).

The O antigen of LPS may be fucosylated and mimic Lewis blood group antigens found on the gastric epithelium. The outer membrane also contains cholesterol glycosides, which are found in few other bacteria. *Helicobacter pylori* has 4-6 lophotrichous flagella; all gastric and enterohepatic *Helicobacter* species are highly motile due to flagella. The characteristic sheathed flagellar filaments of *Helicobacter* are composed of two copolymerized flagellins, FlaA and FlaB (Arthur *et al*., 1999).

2.4 Transmission

The poor hygiene and crowded conditions may facilitate transmission of infection among family members and is consistent with data on intrafamilial and institutional clustering of *H. pylori* infection. Understanding the route of *H. pylori* transmission is important if public health measures to prevent its spread are to be implemented. Iatrogenic transmission of *H. pylori* following endoscopy is the only proven mode. For the general population, the most likely mode of transmission is from person to person, by either the oral-oral route (through vomitus or possibly saliva) or perhaps the fecal-oral route. The person-to-person mode of transmission is supported by the higher incidence of infection among institutionalized children and adults and the clustering of *H. pylori* infection within families. Also lending support to this concept is the detection of *H. pylori* DNA in vomitus, saliva, dental plaque, gastric juice, and feces. Waterborne transmission, probably due to fecal contamination, may be an important source of infection, especially in parts of the world in which untreated water is common (Brown, 2000).

Modes of transmission for *H. pylori* have been identified. However, association studies suggest that there are three other potential routes for the transmission of *H. pylori*: person-to-person transmission (example, oral-oral or fecal-oral), waterborne transmission (example, contaminated water), zoonotic (example, cats and other pets and animals) or vector borne transmission (example, flies). Person-to-person transmission is considered to be the most likely route of transmission considering that isolation of *H. pylori* from non-human reservoirs has been in consistent (Brown, 2000).

2.5 Signs and Symptoms

Although over 80% of individuals infected with the bacterium are asymptomatic, symptoms associated with *H. pylori* can be vague or vary over time. They may be non-specific, or caused by other conditions. Inflammation of, or damage to the stomach lining (gastritis) by *H. pylori* may cause mild or serious reactions to the stomach's contents, stomach ache or abdominal pain, acid reflux, regurgitation, vomiting, belching, flatulence, and nausea. In case of extra digestive dermatological condition such as skin pigment, skin rash, eczema, red knees may appear.

If untreated for a long time, *H. pylori* infections may be related to several serious illnesses: Gastroesophageal reflux disease (GERD), peptic ulcers (duodenal or gastric ulcers), and cancers of the esophagus and stomach (Ierardi *et al*., 1998).

Patients, who have an ulcer or gastritis, may have some of the following symptoms:

Abdominal pain , Bloating and fullness, Dyspepsia or indigestion, Feeling very hungry 1 to 3 hours after eating and Mild nausea (may be relieved by vomiting)

2.6 Pathogenicity

2.6.1 Gastritis

Helicobacter pylori is also found on the inner surface of the stomach epithelial cells and occasionally inside epithelial cells. It produces adhesions which bind to membrane-associated lipids and carbohydrates and help it adhere to epithelial cells. *H. pylori* produces large amounts of the enzyme urease, molecules of which are localized inside and outside of the bacterium. Urease breaks down urea (which is normally secreted into the stomach) to carbon dioxide and ammonia. The ammonia is converted to ammonium by taking a proton (H^+) from water, which leaves only a hydroxyl ion. Hydroxyl ions then react with carbon dioxide, producing bicarbonate which neutralizes gastric acid. The survival of *H. pylori* in the acidic stomach is dependent on urease. The ammonia produced is toxic to the epithelial cells, and, along with the other products of *H. pylori* including proteases, vacuolating cytotoxin A (VacA), and certain phospholipases damages those cells (Ottemann and Lowenthal, 2002).

2.6.2 Skin Diseases

Pathogenic strains of *Helicobacter pylori* have been shown to activate the epidermal growth factor receptor (EGFR), a membrane protein with a tyrosine kinase domain. Activation of the EGFR by *H. pylori* is associated with altered signal transduction and gene expression in host epithelial cells that may contribute to pathogenesis. It has also been suggested that a C-terminal region of the CagA protein (amino acids 873–1002) can regulate host cell gene transcription independent of protein tyrosine phosphorylation (Vaira *et al*., 1999).

2.6.3 Cancer

Two related mechanisms by which *H. pylori* could promote cancer are under investigation. One mechanism involves the enhanced production of free radicals near *H. pylori* and an increased rate of host cell mutation. The other proposed mechanism has been called a "perigenetic pathway" and involves enhancement of the transformed host cell phenotype by means of alterations in cell proteins such as adhesion proteins. It has been proposed that *H. pylori* induces inflammation and locally high levels of TNF-α and/or interleukin 6 (IL-6) (Guo *et al*., 2011).

2.6.4 Vascular Disease

Various studies have found that the presence of a chronic infection by some microbial species could act as risk factor in vascular disease. In particular, several epidemiological studies have been carried out on the association between ischaemic heart disease (IHD) and *H. pylori* infection (Arthur, *et al*., 1999).

2.6.5 Immunological Disease

Several clinical observations suggest a role for *H. pylori* infection in various immunological disorders. Some reports have shown healing of some autoimmune disease such as (Henoch –Schonlein purpura, Sjogren syndrome, and autoimmune thrombocytopenia) after eradication of *H. pylori*. Furthermore, the observation of complete disappearances of some cases of extragasteric mucose associated with lymphoid tissue (MALT) lymphoma (Owens and Smith, 2011).

2.6.6 Liver and Biliary Tract

A higher prevalence of *Helicobacter pylori* infection has been described in patients with liver cirrhosis than in age and sex matched controls (Andersen and Espersen, 1992).

2.6.7 Other Extra –gastero duodenal Disease

Helicobacter pylori infection is reported to be more highly frequent in patients with sideropenic anaemia compared with healthy controls (Haghi-Ashtiani and Monajemzadeh, 2008).

2.7 Diagnosis

Diagnosis of infection is usually made by checking for dyspeptic symptoms and then doing test which can suggest *H. pylori* infection which is usually agnosed in one of these ways(Kusters *et al*2006) (Table 1-2):

2.7.1 Biopsy

The less desirable invasive approach requires an endoscope examination with biopsy, stomach biopsy taken from the lining of the stomach and duodenum. Several different tests may be done on biopsy. It may be treated with chemical to see if an enzyme that break down urea is present. The presence of enzyme indicates an *H. pylori* infection. The biopsy sample may be stained with special dye and examined under microscope to look for *H. pylori* bacteria. In rare cases, a sample may be placed in container that promotes the growth of *H. pylori* bacteria. Then does sensitivity test to determine which antibiotic should be used to treat the infection. A stomach biopsy is the most difficult and expensive method to test for *H. pylori* infection (Vaira *et al*., 1999).

2.7.2 Urea Breath Test

Alternative non – invasive tests include carbon urea breath test which the bacterium metabolizes producing labeled carbon dioxide that can be detected in the breath. Urea breath test, the patient swallow a capsule or drink water that contain urea, the urea tagged with radioactive carbon (carbon 14). If *H. pylori* bacteria is present in the stomach, they will break down the urea eventually causing patient to exhale carbon that contains the tagged carbon. The breath test is not always available and it is accurate and safe; some drugs can affect *H. pylori* urease activity and give false negative with urea breath test (Vaira *et al*., 1999).

2.7.3 Stool Antigen Test

Stool antigen test includes an antigen detection kit which has been developed for the detection of *Helicobacter pylori* in faeces (Vaira *et al*., 1999).

2.7.4 Serological Examination

Most commercially available serological kits used the enzyme linked immunosorbent assay (ELISA). Originally the kits use crude antigen preparation although many of the newer kits use a more purified antigen preparation. patient test kits are based either on latex agglutination or immunochromatography (ICT.) Generally they have low sensitivity compared with laboratory tests. Western blotting, ELISA, and recombinant immunoblot assay (RIBA) have also been developed into commercially available kits and can be used to indicate the presence of specific virulence markers.

Immunological reagents have also been combined with other diagnostic modalities to develop immunohistochemical stains and DNA immunoassay. Serology is an important method of determining colonization status and can be used for diagnosis, as screening procedure, or to follow the efficacy of eradication regimens. Most assays detect IgG in serum although some detect IgA. More recently developed

assay detect IgA in saliva and the production of affinity purified antibodies has led to the development of an antigen detection assay for faecal specimens. Serological reagents have also been used in immunocytochemistry and to speed up the detection of amplified products of the polymerase chain reaction (PCR) (Vaira *et al*., 1999).

Table (2): Diagnosis of *H. pylori* infection

Diagnostic method	Sensitivity and specificity	Typical application	Remarks
Invasive methods			
Histology	>95%	"Gold standard" in routine hospital diagnostics	Requires expert pathologist; also provides histological data on inflammation and atrophy
Culture biopsy	>95%	Alternative gold standard	Allows for testing of antimicrobial sensitivity; requires specific microbiological expertise
Rapid urease test	>90%	Cost-effective and rapid test	Requires an additional test for confirmation of *H. pylori* infection
Noninvasive methods			
Urea breath test	>95%	Alternative gold standard	Very useful, reliable test to evaluate success of eradication treatment of *H. pylori*; limited availability due to requirement of expensive equipment
Fecal antigen test	>90%	Not widely used yet	Simple test but may not be reliable for evaluation of success of eradication treatment of *H. pylori*
Serology	80-90%	Mainly used for epidemiological studies	Insufficient reliability for routine screening; cannot prove ongoing infection due to immunological memory

2.8 Immunity

After contact with *H. pylori,* IgA, IgG, and IgM antibodies can occur in the serum .The specific IgM disappears after a few weeks. IgA antibody can still be detected after a considerable period. Elevated IgA titer is found after IgM titer has fallen and can persist over many years. IgA antibody are formed locally, but are not detected in the serum in every case (Kaira *et al*., 2006).

A positive IgA result correlates well with the gastritis activity. An elevated IgA antibody titer is considered to be a marker for gastric infection activity. Antibody against *H. pylori* occurs in about 70% of patient with chronic active gastritis, they are associated with ulcer condition in 60% - 90% of cases .Test of specific IgA antibody against *H. pylori* are suitable indicator for the complete eradication of the agent as a part of therapy monitoring . A significant drop in the IgA antibody titer after about 6week of therapy is a sign of success (Kaira *et al*., 2006).

2.9 Epidemiology

The prevalence of *H. pylori* shows large geographical variations. In various developing countries, more than 80% of the population are *H. pylori* positive, even at young ages. (Perez -Perez *et al*., 2004). The prevalence of *H. pylori* in industrialized countries generally remains under 40% and is considerably lower in children and adolescents than in adults and elderly people (Pounder , 1995). Within geographical areas, the prevalence of *H. pylori* inversely correlates with socioeconomic status, in particular in relation to living conditions during childhood (Fiedorek *et al*., 1991)**.**

In Western countries, the prevalence of this bacterium is often considerably higher among first- and second-generation immigrants from the developing while the prevalence of *H. pylori* infection in developing countries remains relatively constant, it is rapidly declining in the industrialized world. The latter is thought to

be caused by the reduced chances of childhood infection due to improved hygiene and sanitation and the active elimination of carriership via antimicrobial treatment. In developing countries, *H. pylori* infection rates rise rapidly in the first 5 years of life and remain constantly high thereafter, indicating that *H. pylori* is acquired early in childhood (Perez - Perez *et al.*, 2004).

In industrialized countries the prevalence of *H. pylori* infection is low early in childhood and slowly rises with increasing age. This increase results only to a small extent from *H. pylori* acquisition at later age. The incidence of new *H. pylori* infections among adults in the Western world is less than 0.5% per year; the higher prevalence of infection among the elderly thus reflects a birth cohort effect with higher infection rates in the past (Parsonnet, 1995).

2.10 Treatment

Once *Helicobacter pylori* is detected in patients with a peptic ulcer, the normal procedure is to eradicate it and allow the ulcer to heal. The standard first-line therapy is a one week "triple therapy" consisting of a proton pump inhibitors such as omeprazole, lansoprazole and the antibiotics clarithromycin and amoxicillin (Mirbagheri *et al.*, 2006).

An increasing number of infected individuals are found to harbor antibiotic-resistant bacteria. This results in initial treatment failure and requires additional rounds of antibiotic therapy or alternative strategies such as a quadruple therapy, which adds a bismuth colloid, such as bismuth subsalicylate (Megraud and Lamouliatte, 2003).

For the treatment of clarithromycin-resistant strains of *H. pylori* the use of Levofloxacin as part of the therapy has been suggested (Perna *et al.*, 2007). Tetracycline, amoxicillin, imidazoles (predominantly metronidazole and tinidazole), and a few selected macrolides (in particular clarithromycin, sometimes

azithromycin) are probably the drugs most widely used for *H. pylori* eradication therapy (Megraud and Lamouliatte, 2003).

The triple therapy with omeprazole, amoxicillin, and metronidazole failed to eradicate *H. pylori* in the majority of patients, which is an essential argument to withdraw this regimen out of the national recommendations. Macrolide with amoxicillin are preferable to achieve higher eradication rates. Azithromycin (1 g for the first 3 days) can be considered as a successful component of the triple PPI-based regimen (Ivashkin *et al.*, 2002).

The re-treatment with tetracycline combination regimen cured 77.7% of patients: that seems to be a promising option, in clinical practice, after an eradication failure (Auriemma and Signorell, 2001).

In case of treatment of chronic urticaria in children, always keeping in mind those medications may need to be adjusted to meet pediatric requirements (Wieczorek *et al.*, 2004).

2.11 Prevention

Rising antibiotic resistance increases the need for a prevention strategy for the bacteria (Selgrad and Malfertheiner, 2008).

Studies have recently been published suggesting *H. pylori* activity could be suppressed via dietary methods. In 2009, Japanese study in Cancer Prevention Research found eating as little as 70 g (2.5 ounces) of broccoli sprouts daily for two months reduces the number of colonies of *H. pylori* bacteria in the stomach by 40% in humans. This treatment also seems to help by enhancing the protection of the gastric mucosa against *H. pylori*, but is relatively ineffective on related gastric cancers. The previous infection returned within two months after broccoli sprouts were removed from the diet, so an ongoing inclusion in the diet is best for continued protection from *H. pylori* (Yanaka *et al.*, 2009).

In 2008 a study published in Korea it was found that kimchi (fermented cabbage) contains a bacterial strain "showing strong antagonistic activity against *H. pylori*." The bacterium strain isolated from kimchi, designated *Lactobacillus. plantarum* NO1 was found to reduce the urease activity of *H. pylori* by 40-60% and suppress its binding to a human gastric cancer cell line by more than 33% (Lee and Chang, 2008).

In 2009 study has found green tea can prevent *Helicobacter*-related inflammation (Stoicov *et al*., 2009).

Chapter Three

3.Materials and Methods

3.1 Study design

3-1-1 Type of the study

This is a desciptive cross sectional laboratory based study

3-1-2 Study area

The study was carried out in Saudia Arabia (Riyadh).

Interview of the patients and sample collection were done in King Saud Medical City (K.S.M.C)

3-1-3 Study population

Asymptomatic from different nationality Saudi, Sudanese, Egyptian and Bangladesh people including males and females *H. pylori* carrier who visited K.S.M.C

3-1-4 Data collection

Data were collected using a questionnaire (appendix I) with informed consent.

3-2 Ethical Consideration

Volunteers were informed about the importance and benefits of the study.

A written informed consent was taken from patients.

3-3 Inclusion Criteria

Subjects infected with *Helicobacter pylori* but show no symptoms of infection.

3-4 Exclusion Criteria

Subjects with no *Helicobacter pylori* infection or showing symptoms of other disease.

3-5 Sample technique

A Total of 160 stool samples and 160 serum samples were collected and 15 healthy as negative control.

3-5-1 Collection of specimens

The first was stool for *H.pylori* stool Ag .The second specimen was blood (serum) to detect antibody against *H.pylori* antigen by different techniques Enzyme Linked Immunosorbent Assay (ELISA) IgG ,IgA ,Immunoflorsent Test IgG and Western Blot Ig G Technique . All specimens were transported in ice packed box to Khartoum State (Research Laboratory) in Sudan University of Sciense and Technology and preserved in the refrigerator at -20^0 C.

3-6 Sample Collection

3-6-1 Collection of stool samples

A clean dry screw cap container was given to each patients, stool samples were processed as soon as possible .

3-6-2 collection of blood samples

Blood was withdrawn in sterile vacutainer tubes without any anticoagulants. Samples were stored and transported to the laboratory at a temperature between $+2^0$ C and $+8^0$ C .

3-7 *H.pylori* stool antigen

A rapid Immunoassay for detection of *Helicobacter pylori* antigen in stool . IF the stool sample was liquid or semi-solid the red cap was unscrewed from sample diluents vial then 100ul was withdrawn by a clean calibrated transfer pipette.

The mixed sample was dispensed into the sample diluted vial, the liquid stool and the diluent sample were thoroughly but gently by squeezing the pipette bulb three times. The vial was recapped tightly and mixed thoroughly but gently by swiriling the contents of the vial for seconds . A smear portion of solid sample was

transferred by which plastic applicator into the vial .The vial was then hold vertically and gently tapped on the benchtop .then the tip from the vial was broken.Four drops were dispensed into the round window of the device ,results were read after 5 minutes was described by the manufacturer IMMUNOCARD STAT (HPSA) TEST (Meridian Biosciense,inc)

3-8 Enzyme Link Immuno Sorbent Assay (ELISA)

3-8-1 Anti –*Helicobacter pylori* ELISA IgG

ELISA is designed for the detection of immunoglobulin G antibody against *Helicobacter pylori* in human serum . ELISA IgG is screening test used for detection of infection with *Helicobacter pylori* and checking of patients treatment . Moreover ,this test is used for differential diagnosis of chronic phase of disease . In this assay procedure ,purified *H.pylori* antigen was coated on surface of micro wells. Diluted patient samples (Appendix I) was added to the wells and *H.pylori* IgG specific antibody ,if present ,binds to the antigen . All unbound materials were washed away. Enzyme conjugate was added , which binds to the antibody –antigen complex . Excess Enzyme conjugate was washed off and solution of chromogen (substrate reagent) was added .The enzyme catalytic reaction was stopped at a specific time by sulphuric acid.

According to the manufactures protocol 100ul from positive and negative controls or diluted patient samples were transferred into the i microplate wells then incubated for 30 minutes at room temperature. The wells were then emptied and subsequently washed 3 times using 300 ul of wash buffer (AppendixII) for each wash, then 100 ul of enzyme conjugate (peroxide –labelled anti –Human IgG)was added into each of the microplate wells and incubated for 30 minutes at room temperature .The wells were then emptied and subsequently washed 3 times using 300 ul of wash buffer for each wash, then 100 ul was added from TMB substrate

solution (chromogen)into each of the microplate wells and incubated for 15 minutes at room temperature. The reaction was stopped by adding 100 ul of stop solution into each of microplate wells in the same order and at the same speed as the substrate solution was introduced . Photometric measurement of the color intensity was done at a wavelength of 450 nm and a reference wavelength of between 620 nm and 650 nm within 30 minutes of adding the stop solution . Prior to measuring , the microplate was shaking slightly to ensure homogeneity . The results obtained according to the following formula : Index of positivity was calculated by dividing the absorbance of each tested sample by the mean absorbance of cut –off .EUROIMMIUN (Germany)recommends interpreting results as follows:

Ratio< 0.8= negative

Ratio> 0.8 to <1.1 =Borderline

Ratio> 1.1=positive

3-8-2 Anti –*Helicobacter pylori* ELISA (IgA)

Then one Hundred ul were transferred from positive and negative controls or diluted patient samples into the individual microplate wells, and incubated for 30 minutes at room temperature . The wells have been emptied and subsequently washed 3 times using 300 ul of wash buffer for each wash . 100 ul of enzyme conjugate (peroxidase –labelled anti – human IgA) was suspended into each of the microplate wells and incubated for 30 minutes at room temperature .The wells were emptied and subsequently washed 3 times using 300 ul of wash buffer for each wach . 100 ul was added from TMB substrate solution into each of the microplate wells and incubated for 15 minutes at room temperature. Finally, 100 ul of stop solution was added into each of microplate wells in the same order and at

the same speed at the substrate solution . Photometric measurement of the colour intensity was read at a wavelength of 450 nm and a reference wavelength of between 620 nm and 650 nm within 30 minutes of adding the stop solution . Prior to measuring , the microplate was shaked slightly to ensure homogeneity . The results obtained according to the following formula : Index of positivity was calculated by dividing the absorbance of each tested sample by the mean absorbance of cut –off .The result 0.9 was considered as negative while result 1.1 was considered as positive . EUROIMMUN recommends interpreting result as follows ;

Ratio >0.8 negative

Ratio<0.8 to> 1.1= Borderline

Ratio > 1.1 =positive

3-9 Indirect Immunofluorescense Test (I I FT)

3-9-1 Materials of IIFT

The slides were incubated for 15 minutes at room temperature before used .

Fluorescent –Labeled Secondary Antibody mixed thoroughly with conjugate protected from sunlight . Positive and negative control were made ready and mixed thoroughly ,before used . PBS-Tween was prepared in the lab (Appendix ll) . Embedding medium was also prepared . Reagent trays must be hydrophilic and the surrounding area hydrophobic.

3-9-2 IIFT Method (Titer plane Technique)

All reagent and serum samples were prepared by applying 30 ul of diluted sample (Appendix ll) to each reaction field of the reagent tray using polystyrene pipetting template .Then were incubated for 30 min at room temperature ($+18^0$ C

to $+25^0$C) until ensured that each sample contacted with its BIOCHIP and the individual samples do not come into contact with each other . The Biochip slides were rinsed in a flush of PBS-Tween in a cuvette for at least 5 min in a rotary shaker . 25 ul of Fluorscein labelled Anti –Human globulin were applied to each reaction field of a clean reagent tray . One Biochip slide was removed from cuvette within five seconds and blotted only at the back with a towel paper and immediately put into the recesses of the reagent tray . The slides were protected from direct sunlight and incubated for 30 min at room temperature ($+18^0$ C to $+25^0$ C).The cuvette was then washed with new PBS –Tween buffer at least 5 min . Embedding medium was placed onto a cover glass drops of max .10 ul per reaction field and the slides were dried at the back. Then the cover glass was properly fitted into the reassess of the slide . The Fluorescence was read with the microscope objective 20x for tissue sections objective 40x for cell substrates using excitation filter ;488 nm , colour separator ;510nm , blocking filter;520nm ,light source;mercuray vapor lamp ;100w ,(EUROIMMUN LED, EUROSTAR) Blue light .

IF antibodies against *Helicobacter pylori* were present in the sample (positive reaction), a distinct fluorescence of the bacteria covering the reaction area becomes visible . Essentially the same result would be obtained with the positive control serum. Depending on the sample, the fluorescence pattern appears in parts circular or granular were classified to + ,++ ,+++ depending on infection . In the case of a negative result the cells would show no fluorescence.

3-10 WESTERN BLOT (IgG) EUROLINE

The blot strips were blocked and incubated in the first reaction step with diluted patient samples. In the case of positive samples , specific antibodies of the class IgG bound to the antigen .To detect the bound antibodies ,a second incubation

was carried out using an enzyme –labelled anti – human Ig G (Enzyme conjugate) catalyzing a colour reaction.

3-10-1 WB Materials EUROLINE

Coated test Strips

After removal the packing of the strips from refrigerator at 2^0 C to 8^0 C in dry place.The blot strips were printed with a number in addition to the test kit lot number. This number refers to the strip bath and was also printed on the corresponding evaluation template .These two numbers must match to ensure correct evaluation of test result.

Enzyme Conjugate

The Enzyme conjugate was prepared and diluted with 1:10 with universal buffer (Appendix III) ,with deionised or distilled water and should be used only at the same working day and put substrate solution .

3-10-2 Method

3-10-2-1 manual incubation

Each channels of the incubation tray was filled .with 1.5 ml universal buffer (Appendix III) the strips was placed in a channels then incubated . for 15 minutes at room temperature on a rocking shaker . After that , the channel was filled with 1.5 ml of the diluted .serum sample and when incubated at room temperature for 30 minutes on rocking shaker ,The mixture was aspirated off from the channel and washed 3 times with the universal buffer , After a aspiration ,1.5 ml of diluted .1:10 enzyme conjugated was placed in each channel for 30 minutes on shaking .The conjugated was aspirated off and washed 3 times , Then 1.5 ml of substrate solution was transferred into each channel and incubated for 10 minutes of room temperature under shaking . Lastly ,each strip was washed .3 times with deionized water for one minutes .

3-11 Data Analysis

All the data were analyzed using statistical package for social science (SPSS).The obtained data was analyzed in parameters of age , sex ,nationality ,symptoms and chronic disease .chi-square was used to test the significance of infection among the selected patients . Also means, tables and charts were used to explain the results .

3-12 Calculation of Sensitivity and Specificity

The sensitivity and specificity of the tests used were calculated according to the formula

Sensivity=(True positive /(True positive +False negative))*100%

Specificity =(True negative /(False positive +True negative))*100%

Positive Predictive Value (PPV)=(True positive /(True positive False positive))*100%

Negative Predictive Value (NPV) =(True Negative /(True Negative False Negative))*100%

Accuracy = (true Positive + True Negative)/ total sample)*100%

CHAPTER FOUR

4. RESULTS

4.1. DISTRIBUTION OF SAMPLES

The study samples included 85 males and 75 females (n=160), 101 patients (63.1%).were more than 30 years old The distribution according to nationality was 10 (6.3%)Bangladeshi,11 (6.9%) Sudanese, 17(10.6%) Egyptians, and 122 (76.3%) Saudi(Table 3).

Table3. Distribution of samples according to gender ,age and nationality (N= 160)

Variable	**n (%)**
Gender	
Males	85 (53.1)
Females	75 (46.9)
Age (years)	
≤18	15 (9.4)
19-30	44 (27.5)
31-45	48 (30.0)
> 45	53 (33.1)
Nationality	
Bangladeshi	10 (6.3)
Sudanese	11 (6.9)
Egyptians	17 (10.6)
Saudi	122 (76.3)

4. 2. Prevalence of *H. pylori* Infection by Different Tests

Table 4 and Fig 1 show the results of stool antigen (HpSA) test which depict the prevalence *H. pylori* infection; 76/160 (47.5%) of the samples were infected and 84/160(52.5) were not infected by *H. pylori*.

Most of the study samples were diagnosed to have *H. pylori* infection by using ELISA (IgG) test 153 (95.69%) (Fig 2). On the other hand, ELISA (IgA) test showed that 80/160 (50%) were positive, 37/160 (34.4%) were negative and 25/160 (15.6%) were borderline (Fig 3) (Table 5).

Table 6 and Fig 4 showed Immunofluorescence IgG test, out of 59 participants, there were 46 (77,7 %) positive , while the rest of the samples were positive , 10 infected by *H. pylori* with low load of the antibodies, 20 have more antibodies and more serious infection and 16 have a disease with dangerous and high risk.

Table 7 and Fig 5 showed the results of western blot test (n=30), 17(56.7%) were positive and 13 (43.3%)were negative.

Table 4 . The prevalence of *H. pylori* infection among study sample using stool antigen (HpSA) test (n=160)

Test	n (%)	
	Infected (positive)	**Non infected (negative)**
Stool antigen	76(47.5)	84(52.5)

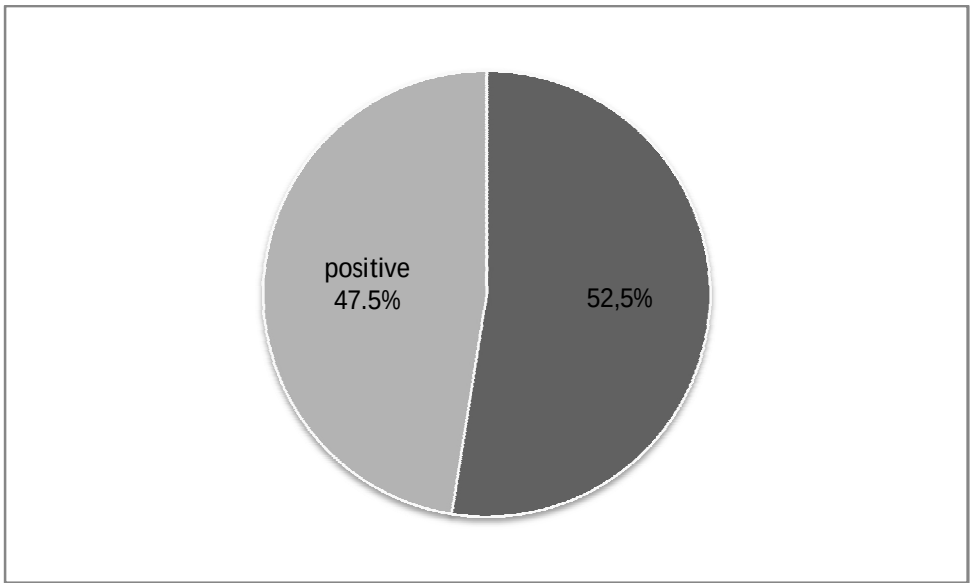
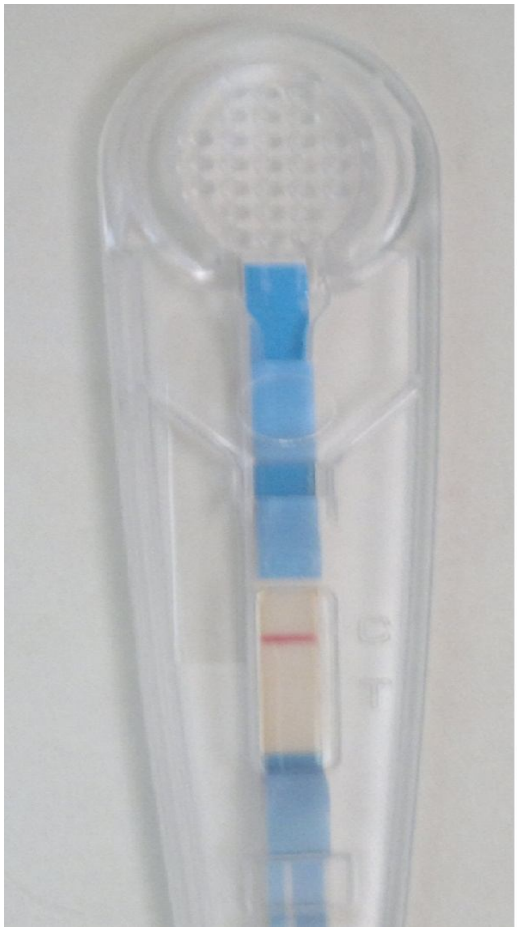
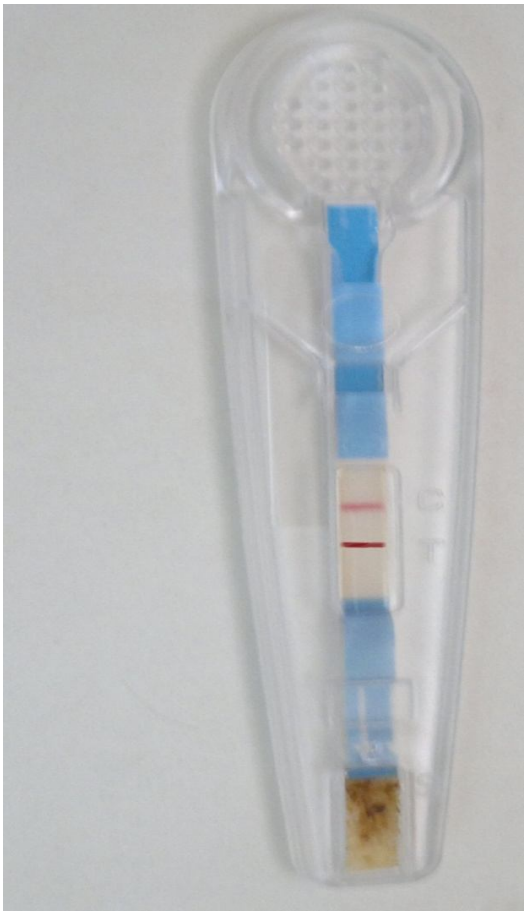


Fig1. The prevalence of *H. pylori* infection among study samples using stool antigen test .



1



2

Fig (6)

1.Helicobacter pylori antigen in stool (Negative)one line red colour in control only .

2. .*Helicobacter pylori* antigen in stool *(*Positive*)* two lines red colour in control and test .

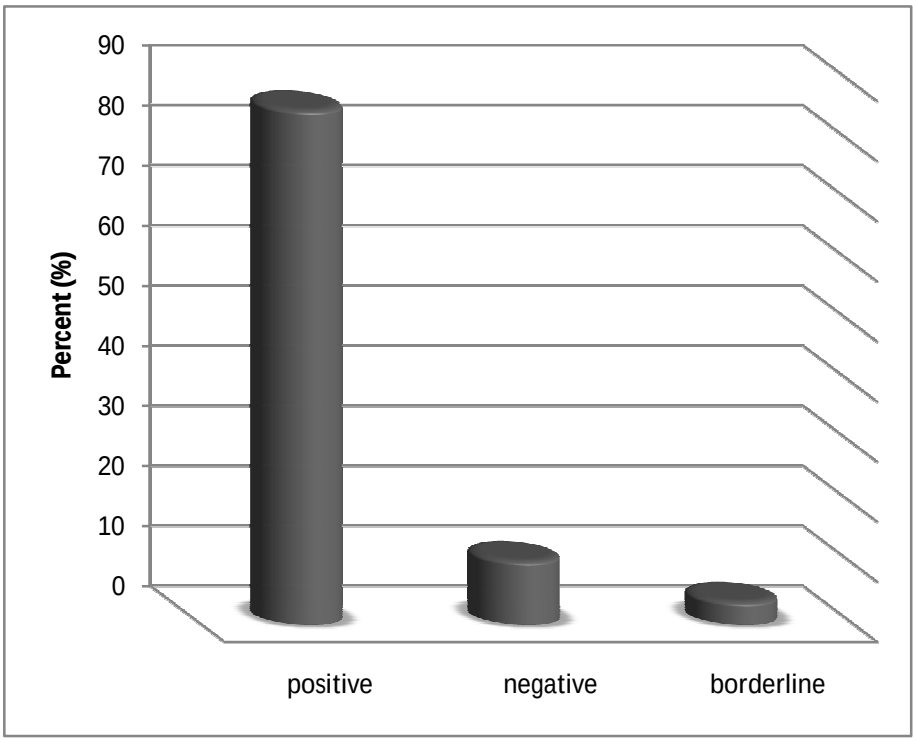


Fig 2. The prevalence of *H. pylori* infection among study samples using ELISA (IgG) test .

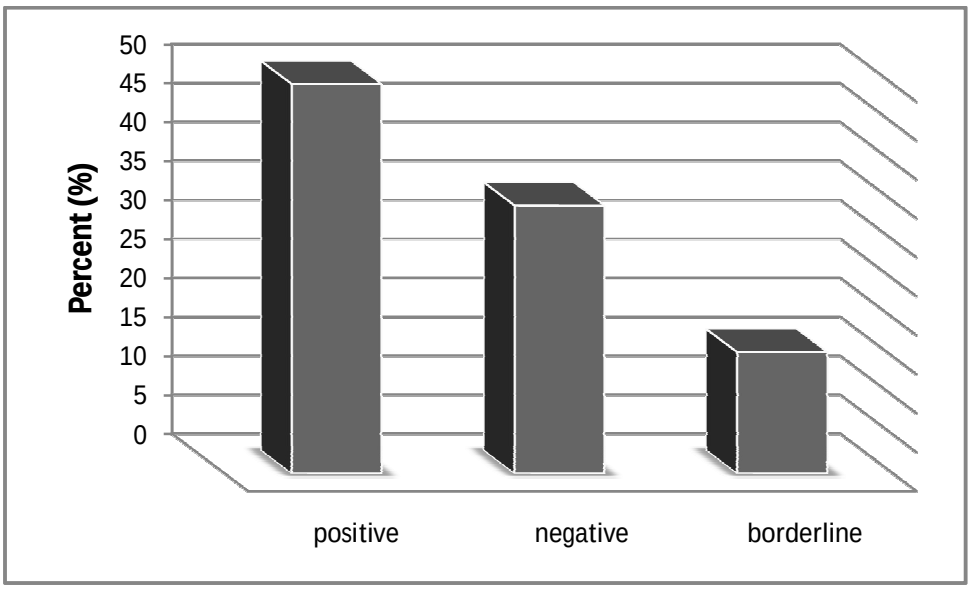


Fig3. The prevalence of *H. pylori* infection among study sample using ELISA (IgA) test.

Table 5 . The prevalence of *H. pylori* infection among study sample using ELISA (IgG) test and ELISA test (IgA) .

Test	Results n (%)		
	Infected (positive)	Non infected (negative)	Borderline
ELISA (IgG)	137 (85.6%)	17 (10.6%)	6 (3.8%)
ELISA (IgA)	80 (50.0%)	37 (34.4%)	25 (15.6%)

Table6.
IgG intensity of *H.pylori* infection among study sample using Immunofluorescence IgG test .

Immunofluorescence IgG Test	Results n (%)	
	Infected (positive)	**Non infected (negative)**
	+	-
	46 (77,7%)	13 (22.0%)

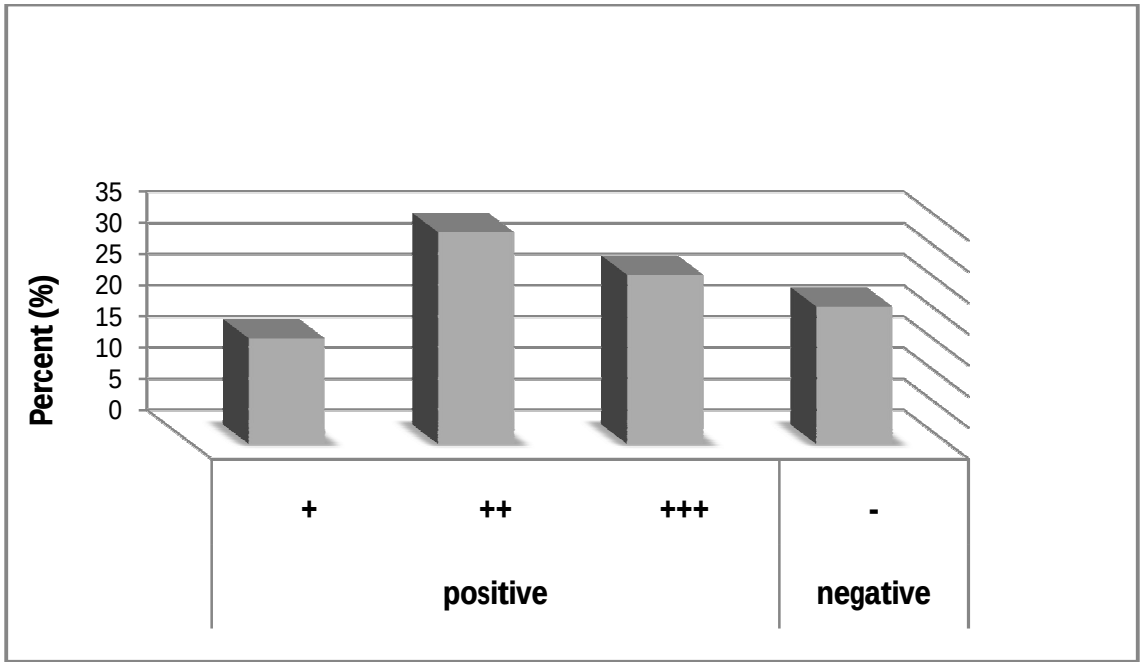


Fig 4. The prevalence of *H. pylori* infection among study sample using Immunofluorescence IgG test (n=59). + Low load of the antibodies, ++ more antibodies and more serious infection; +++ disease is dangerous and high risk.

Table7. The prevalence of *H. pylori* infection among study sample using western blot test (n=30)

Test	**n (%)**	
	Infected (positive)	**Non infected (negative**
western blot	17(56.7)	13 (43.3)

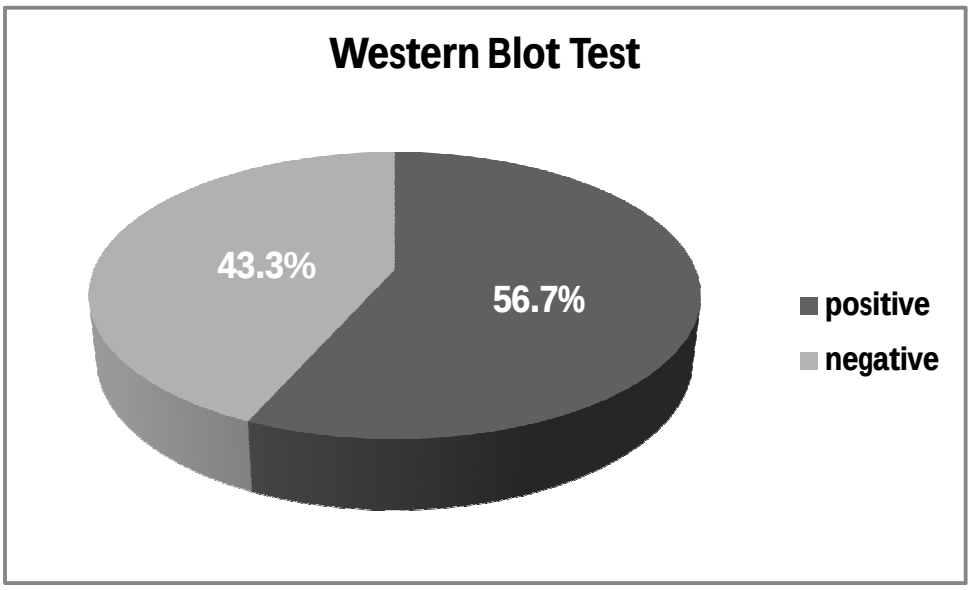


Fig5. The prevalence of *H. pylori* infection among study sample using Western blot

4.3. The Relationship of *H. pylori* Infection with study samples

Tables 8 to 11 demonstrate the association between *H. pylori* infection diagnosis with different tests and the study samples . The results of stool antigen test showed that the majority of males and females were negative (52.9%, 52.0% respectively). Participants age and nationality did not show significant relationship with *H. pylori* infection (p>0.05). Most of Bangladeshi and Sudanese showed low *H. pylori* infection (table 8).

Table 9 and 11 show the distribution of the sample with *H. pylori* infection diagnosis with ELISA (IgG) and ELISA (IgA) according to gender, age, and nationality were not associated with the *H. pylori* infection diagnosis with ELISA (IgG) and ELISA (IgA) P> 0.05.

Table 11 presents the association of samples with *H. pylori* infection and diagnosis with Immunofluorescence IgG according to gender, age and nationality. The probability of being infected by *H. pylori* differs by sex, and was higher for female. Moreover, this probability increase significantly by age (p= 0.009); a greater percentage of infected participants (positive result) were aged more than 30 years. The differences among age categories with the infections were significant (p=0.009). The nationality demonstrated that those who were from Egypt were more likely to be classified to have *H. pylori* infection.

With regard to western blot test, participants age seemed to be associated with *H. pylori* infection (p=0.028). Participants aged more than 45 were more likely to be infected with *H. pylori*, while participants aged less than 30 were more likely to be with negative results. On the other hand, gender and nationality showed no association with *H. pylori* infection (p= 0.785, 0.642 respectively). (Table 12).

Table8. The Relationship of *H. pylori* infection diagnosed tested by stool antigen test with participants' characteristics

Variables	Positive	Negative	Total	P-value
	N (%)	N (%)		
Gender				0.905
Male	40 (47.1)	45 (52.9)	85	
Female	36 (48.0)	39 (52.0)	75	
Age (years)				0.753
≤18	8(53.5)	7(46.7)	15	
19-30	22(50.0)	22(50.0)	44	
31-45	24(50.0)	24(50.0)	48	
> 45	22(41.5)	31(58.5)	53	
Nationality				0.499
Bangladeshi	4(40.0)	6(60.0)	10	
Sudanese	3(27.3)	8(72.7)	11	
Egyptians	9(52.9)	8(47.1)	16	
Saudi	60(49.2)	62(50.8)	120	

Table 9: The Relationship of *H. pylori* infection diagnosed tested by ELISA (IgG) with participants' characteristics .

Variables	Positive	Negative	Borderline	Total	P-value
	N (%)	N (%)	N (%)		
Gender					0.148
Male	70 (82.4)	11 (12.9)	4(4.7)	85	
Female	67 (80.2)	6 (8.0)	2(2.7)	75	
Age (years)					0.453
≤18	11(73.3)	3(20.0)	1(6.7	15	
19-30	35(79.5)	8(18.2)	1(2.3)	44	
31-45	46(95.8)	1(2.1)	1(2.1)	48	
> 45	45(48.9)	5(9.4)	3(5.7)	53	
Nationality					0.389
Bangladeshi	9(90.0)	1(10.0)	0(0)	10	
Sudanese	9(81.8)	2(18.2)	0(0)	11	
Egyptians	15(88.2)	0(0)	2(11.8)	16	
Saudi	104(85.2)	14(11.5)	4(3.3)	120	

Table10. The Relationship of *H. pylori* infection diagnosed tested by ELISA (IgA) test with participants' characteristics

Variables	Positive	Negative	Borderline	Total	P-value
	N (%)	N (%)	N (%)		
Gender					0.659
Male	40(47.1)	30 (35.3)	15(17.6)	85	
Female	40 (53.3)	25 (33.3)	10(13.3)	75	
Age (years)					0.390
≤18	4 (46.7)	6(40.0)	2(13.3)	15	
19-30	18(40.9)	21(47.7)	5(11.4)	44	
31-45	28(58.3)	12(25.0)	8(16.7)	48	
> 45	27(50.0)	16(32.2)	10(18.9)	53	
Nationality					0.213
Bangladeshi	4(40.0)	2(20.0)	4(40.0)	10	
Sudanese	4(36.4)	6(54.5)	1(9.1)	11	
Egyptians	7(41.2)	8(47.1)	2(11.8)	16	
Saudi	65(53.3)	39(32.0)	18(14.8)	120	

Table 11: The Relationship of *H. pylori* infection diagnosed testeds by Immunofluorescence IgG with participants' characteristics .

Variables	Positive	Negative	Total	P-value
	N (%)	N (%)		
Gender				0.645
Male	25 (75.8)	8 (24.2)	33	
Female	21 (80.8)	5 (19.2)	26	
Age (years)				0.009
≤18	2 (50.0)	2 (50.0)	4	
19-30	11 (57.9)	8 (42.1)	19	
31-45	18(100.0)	0(0.0)	18	
> 45	15(83.3)	3(16.7)	18	
Nationality				0.657
Bangladeshi	2(66.7)	1(33.3)	10	
Sudanese	4(66.7)	2(33.3)	6	
Egyptians	3(100.0)	0(0.0)	3	
Saudi	37(78.7)	10(21.3)	47	

Table12. The Relationship of *H. pylori* infection diagnosed tested by Western blot with participants' characteristics

Variables	Positive	Negative	Total	P-value
	N (%)	N (%)		
Gender				0.785
Male	10(58.8)	7(41.2)	17	
Female	7(53.8)	6 (46.2)	13	
Age (years)				0.028
≤18	0(0.0)	2 (100.0)	2	
19-30	3(30.0)	7(70.0)	10	
31-45	6(85.7)	1(14.3)	7	
> 45	8(72.7)	3(27.3)	11	
Nationality				0.642
Bangladeshi	1(50.0)	1(50.0)	2	
Sudanese	2(50.0)	2(50.0)	4	
Egyptians	2(100.0)	0(0.0)	2	
Saudi	12(54.5)	10(45.5)	22	

4.4. The Relationship between of *H. pylori* Infection Diagnosis with Different Tests and Clinical Characteristics of the Study Samples.

Table 13 shows the association between *H. pylori* infection diagnosis with different tests and symptoms of the study samples. With regard to stool antigen test there were no significant difference between patients who have symptoms or not.

Also there were no significant difference *H. pylori* infection diagnosis with ELISA (IgG) or ELISA (IgA) and asymptomatic or symptomatic healthy population.

According to Immunofluorescence IgG test and Western blot test, there was no significat association between of *H. pylori* infection and symptoms related to infection.

Table 14 demonstrates that there were no significant relationship between *H. pylori* infection diagnosis with any test and chronic disease of the participants.

Table13. The Relationship of *H. pylori* infection diagnosed tested by different with patients' symptoms

Test	Symptom		Total	P-value
	yes	No		
	N (%)	N (%)		
Stool Antigen				0.286
Positive	6(7.9)	70(92.1)	76	
Negative	11(13.1)	73 (86.9)	84	
ELISA (IgG)				0.723
Positive	15(10.9)	122 (89.1)	137	
Negative	1(5.9)	16(94.1)	17	
Borderline	1(16.7)	5(83.3)	6	
ELISA (IgA)				0.106
Positive	12(15.0)	68(85.0)	80	
Negative	2(3.6)	53(96.4)	55	
Borderline	3(22.0)	22(88.0)	25	
Immunofluorescence IgG test*				
Positive	5(10.9)	41(89.1)	46	0.738
Negative	1(7.7)	12(92.3)	13	
Western blot test**				0.201
Positive	2(11.8)	15(88.2)	17	
Negative	0(0.0)	13(100.0)	13	

*N=59, **N= 30

Table14. The Relationship of *H. pylori* infection diagnosed tested by different with patients' chronic disease

Test	Chronic Disease		Total	P-value
	yes	No		
	N (%)	N (%)		
Stool Antigen (n=160)				0.419
Positive	20(26.3)	56(73.7)	76	
Negative	27(32.1)	57 (67.9)	84	
ELISA (IgG) (n=160)				0.824
Positive	39 (28.5)	98 (71.5)	137	
Negative	6(35.3)	11(64.7)	17	
Borderline	2(33.3)	4(66.7)	6	
ELISA (IgA) (n=160)				0.295
Positive	28(35.0)	52(65.0)	80	
Negative	13(23.6)	42(76.4)	55	
Borderline	6(24.0)	19(76.0)	25	
Immunofluorescence IgG test(n=59)				
Positive	13(28.3)	33(71.7)	46	0.738
Negative	4(30.8)	9 (69.2)	13	
Western blot test (n=30)				0.638
Positive	8(47.1)	9(52.9)	17	
Negative	5(38.5)	8(61.5)	13	

*N=59, **N= 30

4.5 Comparison of Different Test Results for Detection of *H. Pylori* Infection

Table 15 demonstrates the distribution of different tests method for detection of H. pylori infection according to gold standard .

Stool antigen test detected 76 true-positive, no false-positive, 37 false-negative, and 47 true negative patients. The Pylori stat ELISA for IgG detected 112 true-positive, 31 false-positive, 1 false-negative, and 16 true negative participants. Moreover, 93 true-positive, 12 false-positive, 20 false-negative, and 35 true negative persons were detected by ELISA IgA test.

59 participants were selected randomly for Immunofluorescence IgG test, the results showed that 43 true-positive, 3 false-positive, 20 false-negative, and 13 true negative healthy samples. While western blot test identified that 17 true-positive, no false-positive, no false-negative, and 13 true negative healthy samples.

Table15.The Distribution of different tests method for detection *H.Pylori* infection according to gold standard.

TEST	GOLD STANDARD *			
	POSITIVE (N)		NEGATIVE (N)	
	TRUE POSITIVE	FALSE NEGATIVE	FALSE POSITIVE	TRUE NEGATIVE
STOOL ANTIGEN (N=160)	76	37	0	47
ELISA (IGG) (N=160) **	112	1	31	16
ELISA (IGA) (N=160) **	93	20	12	35
IMMUNOFLUORESCENCE IGG TEST (N=59)	43	0	3	13
WESTERN BLOT TEST (N=30)	17	0	0	13

* (The positive result referied to participants who have two or more tests were positive) (Thijs *et al* ., 1996).

**Borderline was consider as positive

Table 16 showed the sensitivity, specificity, accuracy, and positive and negative predictive values for the five tests used in this study. For stool antigen test were 67.3, 100, 71.3,100, and 55.95%, respectively. The respective values for the ELISA for IgG were 99.12, 34.04, 80.0, 78.3, and 94.1%.

Sensitivity, specificity, accuracy, and positive and negative predictive values, respectively, were as follows: 82.3, 74.5, 80, 88.6, and 63.6%.

For Immunofluorescence IgG the results show that the sensitivity were (100%), specificity (81.1%), accuracy (94.9%), positive predictive value (93.5%), and negative predictive value (100%). On the other hand, western blot test showed the same percent for sensitivity, specificity, accuracy, and positive and negative predictive values (PPV and NPV) which were 100%.

Furthermore, table 17 confirms that immunofluorescence IgG and western blot tests had sensitivities, specificities, accuracy, and predictive values >80%.

Table 16. Comparison of different test results for detection of *H .Pylori* infection

Test	Sensitivity* (%)	Specificity** (%)	Accuracy¥	PPV £ (%)	NPV¶ (%)
Stool Antigen	67.26	100.0	71.3	100.0	55.95
ELISA (IgG)	99.12	34.04	80.0	78.32	94.12
ELISA (IgA)	82.30	74.47	80.0	88.57	63.64
Immunofluorescence(IgG) test (n=59)	100.0	81.25	94.9	93.48	100.0
Western blot test (n=30)	100.0	100.0	100.0	100.0	100.0

CHAPTER FIVE
5. DISCUSSION

5.1. DISCUSSION

The present study investigated the prevalence and risk factors associated with *H. pylori* infection. 53.1% of sample from male while 46.7% where from female age ranged from 4 to 74 years.

In this study the overall seropostive of of *H. pylori* infection were different according to the method used; stool antigen 47.5%, ELISA IgG (85.6%),ELISA IgA(50.0%), Immunofluorescence IgG (77.9%) and Western blot (56.7%). These findings are in agreement with Al-Moagel *et al* (1990) .

However, age was significantly associated with infection with *H. pylori* ($P<0.05$). Many studies supported that seropositivity gradually increased with age (Malaty *et al.*, 1996; Khan and Ghazi, 2007; Marie, 2008; Jafar *et al.*, 2013). Khan and Ghazi (2007) conducted a study to find the seroprevalence of *Helicobacter pylori* antibodies among 396 asymptomatic healthy populations in Makkah, Saudi Arabia; they found that subjects who were older than 40 years had higher antibody response than those with lower age. Al-Moagel *et al* (1990) confirmed that older people were at high risk for *H. pylori* infection than younger people.

There was no significant differense to *H. pylori* infection in gender or nationality ($p >0.05$), these results were in agreement with the results of Bakka and Salih

(2002) who reported that *H. pylori* prevalence in healthy people in Libya was the same for females and males .

In contrast numerous studies have showed a positive correlation between gender and *H. pylori* infection (Khan and Ghazi, 2007; Marie, 2008; Jafar *et al.*, 2013). In Saudi Arabia, Khan and Ghazi (2007) found that the *H. pylori* infection was lower in males than females at the age group 20-50 years. In 2008, a study aimed to determine the seroprevalence of *H.pylori* antibodies in 5782 asymptomatic partiapants age from 2 to 82 years symptomatic people in an Urban Area of Saudi Arabia, the results showed that females were more susceptible to *H. pylori* infection than males (Marie,2008)

In this study most of patients infected with *H. pylori* have no symptoms. Many studies supported that there were no signs for *H. pylori* infection in healthy subjects (Ching and Wong, 1999; CDC, 1998; Kalach *et al.*, 2005). The results indicated that there were no significant relation between *H. pylori* infection and chronic disease such as diabetes, hypertension or cardiovascular disease, on the contrary Tanriverdi (2011) found that more than half of diabetic patients infected by *Helicobacter pylori* (56.9%) and there was an association of *H. pylori* and micro albuminuria due to the systemic inflammatory response that lead to progression of diabetic nephropathy (Tanriverdi , 2011). Similar finding was reported by Stone *et al.*, (2001) , who reported that there was a correlation

between Cag A seropositivity and either incident ischemic heart disease or ischemic heart disease mortality.

In Taiwan a study was aimed to investigate the correlation between *H pylori* infection and obesity, It was found that 43.7% % of obese patients have lower *H. pylori* infection compared with control group ($p<0.05$) (Wu *et al.,* 2005). The sensitivity and specificity of fecal antigen were approximately 67% and 100% respectively which were similar to the present studies (Monteiro *et al*., 2001; van Doorn *et al.,* 2000)

In the current study, IgG was detected in 70% of the study samples by ELISA while IgA was detected .In only 50% ,However ,47.5% of the faecal samples were positive. Similar findings was found by Karadeniz *et al.,(*2000) who reported that the positive results were higher for ELISA (IgG) test (67.7%) compared with stool antigen test (22.6%). The sensitivity for ELISA (IgG) test was higher than ELISA (IgA) test , these were in agreement with many studies (Best *et al.,* 1994; She *et al.,* 2009), this might be due to high *H. pylori* –specific serum of IgG titers then the serum of specific IgA (Luzza *et al.,*1995).

Recently, in Saudi study which aimed to determine the *H. pylori* infection in patients using HpSA, and *H. pylori* (IgG, IgM and IgA, the results showed that

the sensitivity, specificity and accuracy of the stool antigen test gave highest values between these tests, this was due to the nature of the patients , who were infected with *H. pylori* (Bedir2012). Furthermore, in this study the sensitivities, specificities, accuracy, and predictive values for immunofluorescence IgG and western blot tests were very high, this is in line with the study of Leal *et al.*, (2008) , who showed that western blot test is one of the most reliable tests for the diagnosis of *H. pylori* infection .

5.2.conclusion

The prevalence of *H. pylori* infection among healthy subjects in Saudi Arabia was high; the prevalence was related significantly to age.

ELISA (IgG) test showed high sensitivities, specificities, accuracy, and predictive values compared with stool antigen and ELISA (IgA) test.

Western blot was useful in the diagnosis *H.pylori* infection since the sensitivities, specificities, accuracy, and predictive values were very high compare with other tests.

It was concluded from this study that the prevalence of H.pylori infection among healthy people in Saudi Arabia was high and it was ngnifically correlated with age ELISA ,IIFT ,western blot and stool antigen.

5.3 Recommendations:

A nationwide epidemiological study is needed to determine the seroprevalence of *H. pylori* in Saudi Arabia.

1. High number of samples is needed to verity these result.

2. Western blot test and ELISA (IgG) are the most reliable tests for the diagnosis of *H. pylori* infection, but because western blot is expensive and need technical demands, ELISA might be more suitable for use in developing countries.

3. More studies about the risk factors and mode of transmission of *H. pylori* are needed to determine the extent of this disease .

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APPENDIX I

ELISA Sample Dilution

Diluted 1:101 in sample buffer

Serum ……. 10 ul

Sample buffer …….. 1.0ml

Mix well by vortexing.

ELISA Buffer

Corbonate bicoronate buffer (Coating buffer) PH:6.9

-NaCO3 anhdrous ……1.59gm

-NaHCO3…….2.93gm

Dissolve in liter D.W .Adjust PH to by NaOH or HCL

Phosphate buffer saline (PBS)/Teen-20 (washing buffer)PH7.4

-NaCL……8gm

-Na2HPO4……2.9gm

-KH2PO4……..0.2gm

-KCL……0.2gm

Dissolve in liter distilled water then add 0.01 V/V polyoxy ethylene sorbitol monourate (Teen-20).Adjust PH by NaOH or HCL.

H2SO4(Stopping buffer)

20%H2SO4

-20 ml ……..H2SO4

-80ml……..D.W

APPENDIX II

Indirect Immunofluorescence test IgG (IIFT)

PBS-Tween:

In 1 pack salt for PBS dissolved in

D.W	1 Liter
Tween-20	2ml

Mixed for 20 min until homogeneous .

Dilution Sample :

1:100 in PBS Tween

Sample	10.1ul
PBS-Tween	1000ul

Mixed thoroughly for 4 seconds

APPENDIX III

EUROLINE-WB(IgG)

Dilution of Enzyme Conjugate:

Ready for use enzyme conjugate the amount required should be removed from bottle

1:10 with universal buffer

Anti –human IgG	0.15ml
Universal buffer	1.35ml

Dilution of Universal buffer:

Ready for use universal buffer the amount required.

Diluted 1:10 with deionised or distilled water

Buffer	1.5ml
Deionised or Distilled water	13.5ml

Dilution Patient Sample:

Diluted 1:51 in diluted universal buffer

Sample	30ul
Diluted Universal Buffer	1.5

APPENDIX IV

Specificity of the antigens source for the :-

EUROIMMUN Anti *Helicobacter Pylori*

EUROLINE –WB is provided by the *Helicobacter Pylori* strain (ATCC 43504), ON The test strips :

Band	**Antigen**	**Specificity**
120 kDa	CagA,P120	Cytotoxin A-associated protein ,high specificity.
95 kDa	VacA P95	Vacuollizing cytotoxin A, high specificity.
75 kDa	P 75	Unspecific
67 kDa	FSH,p 67	Flagellar Sheath Protein .Unspecific ,because of cross reactivity to other bacteria.
66 kDa	UreB, P 66	Heavy urease subunit ,cross reactivity to other bacteria having urease.
57 kDa	HSP homolog , p 57	Heat Shock Protein homolog,unspecific
54 kDa	Flagellin, p 54	Flagellin, unspecific because of cross reactivity to other bacteria having flagella.
50 kDa	P 50	Unspecific.
41 kDa	P 41	Unspecific
33 kDa	P 33	Probably specific
30 kDa	OMP ,P 30	Out Membran Protein ,species specificity.
29 kDa	Ure A,P 29	Light urease subunit ,high specificity.
26 kDa	P 26	High specificity
19 kDa	OMP ,P 19	Outer Membran Protein ,species specificity .
17 kDa	P 17	Probably specific .

Specificity of the antigen :*Helicobacter pylori* antigens can generally be divided into three categories .

Category	Antigens
1	Cross –reacting and undefined antigens with the molecular weight 41 kDa .50 kDa ,54 kDa ,57 kDa ,67 kDa and 75 kDa .
2	Antigen with a molecular weight of 66 kDa (urease B)
3	Species –specific and highly specific antigens with the molecular weights 17 kDa ,19 kDa ,26 kDa ,29 kDa ,30 kDa ,33 kDa VacA and CagA

Interpretation of IgG antibody results:

The results of the *Helicobacter pylori* –WB test can be divided into negative , borderline and positive results .In order to evaluate the signals , the band positions and intensity of staining must be taken into consideration ,as negative sera sometimes produce weak signals in individual bands .

Result	Characteristics
Negative	No antigen bands or weak intensities of some antigen bands from categories 1 and 2 or one single weak antigen band from category 3 (antigen in category 3 are shaded grey in the evalution protocol).
Borderline	A distinctive antigen band from category 3 or a minimum of two weak antigen bands from category 3 (antigens in category 3 are shaded grey in the evaluation protocol)
Positive	A minimum of two distinctive antigen bands from category 3(antigens in category 3 are shaded grey in the evaluation protocol).

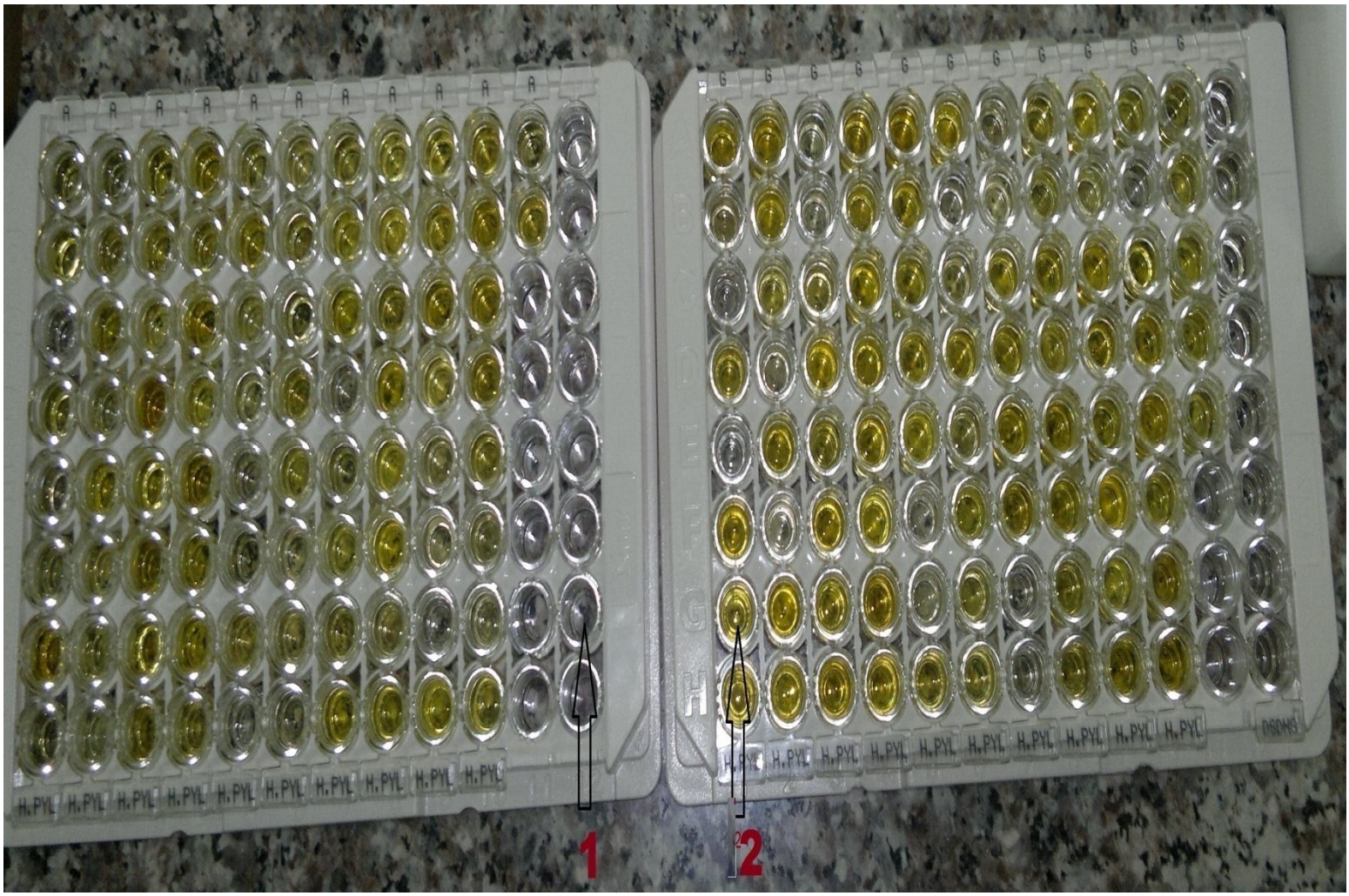


Fig (7):

1. plate shows of ELISA For Antibodies against *Helicobacter pylori* IgG .
2. plate shows of ELISA For Antibodies against *Helicobacter pylori* IgA

YELLOW COLOUR +ve

WHITE COLOUR -ve+

+

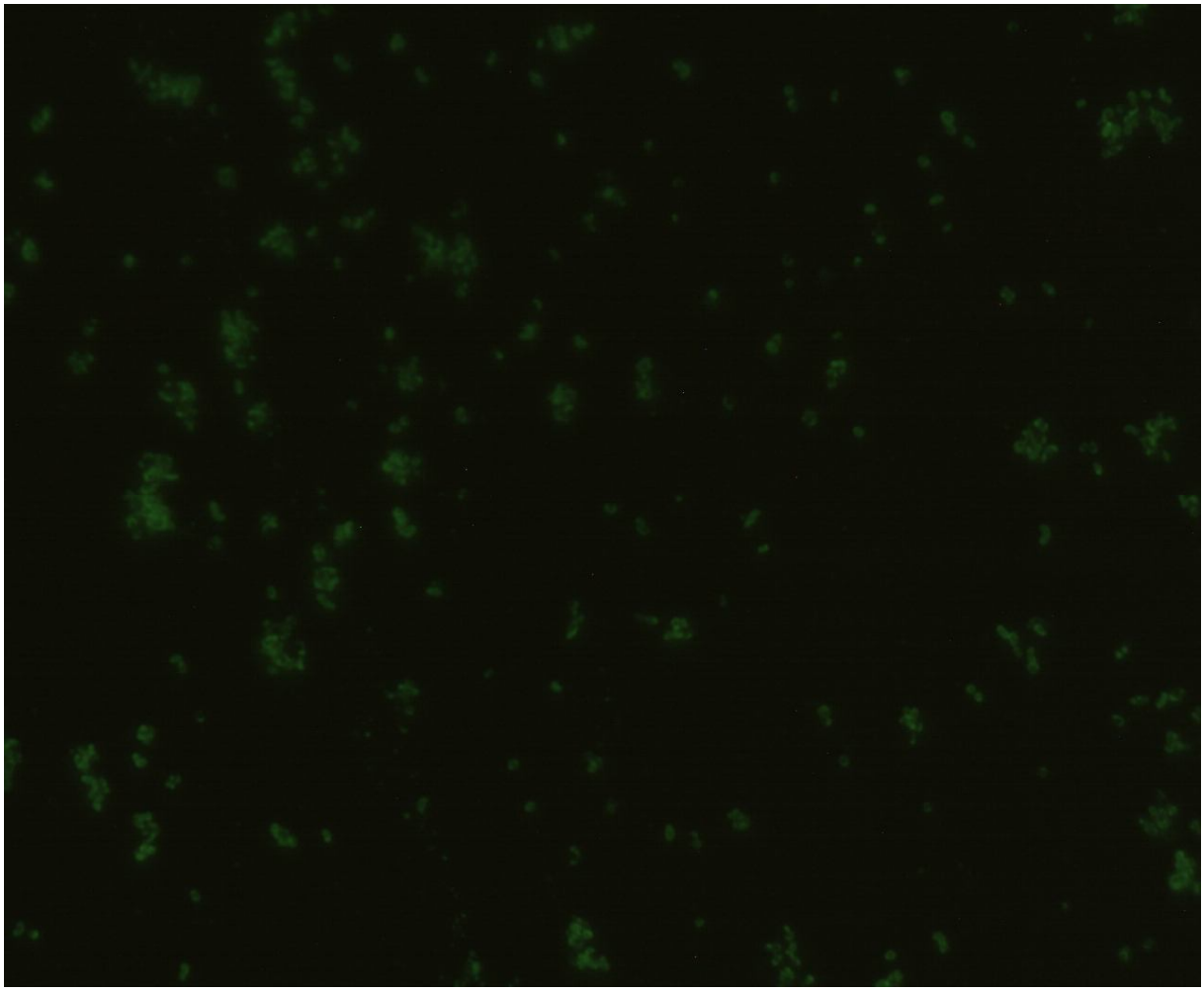


Fig (**8**) Immunofluorescence showing +

These Image shows (Few infection) of the fluorescence pattern appears in parts circular or granular under fluorescence microscope .

Antibodies against *Helicobacter Pylori* IgG are present .

++

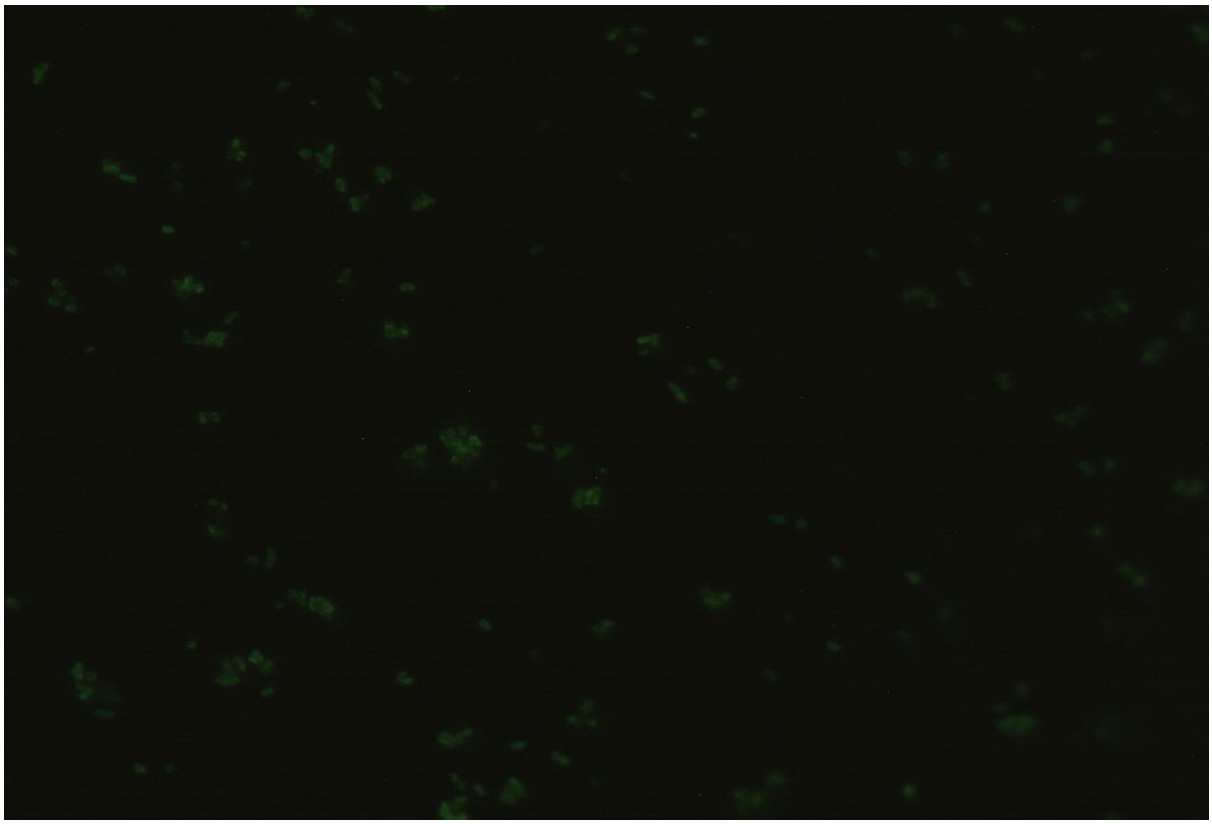


Fig (**9**) Immunofluorescence showing ++

These Image shows (Medium infection) of the fluorescence pattern appears in parts circular or granular under fluorescence microscope .

Antibodies against *Helicobacter Pylori* IgG are present .

+++

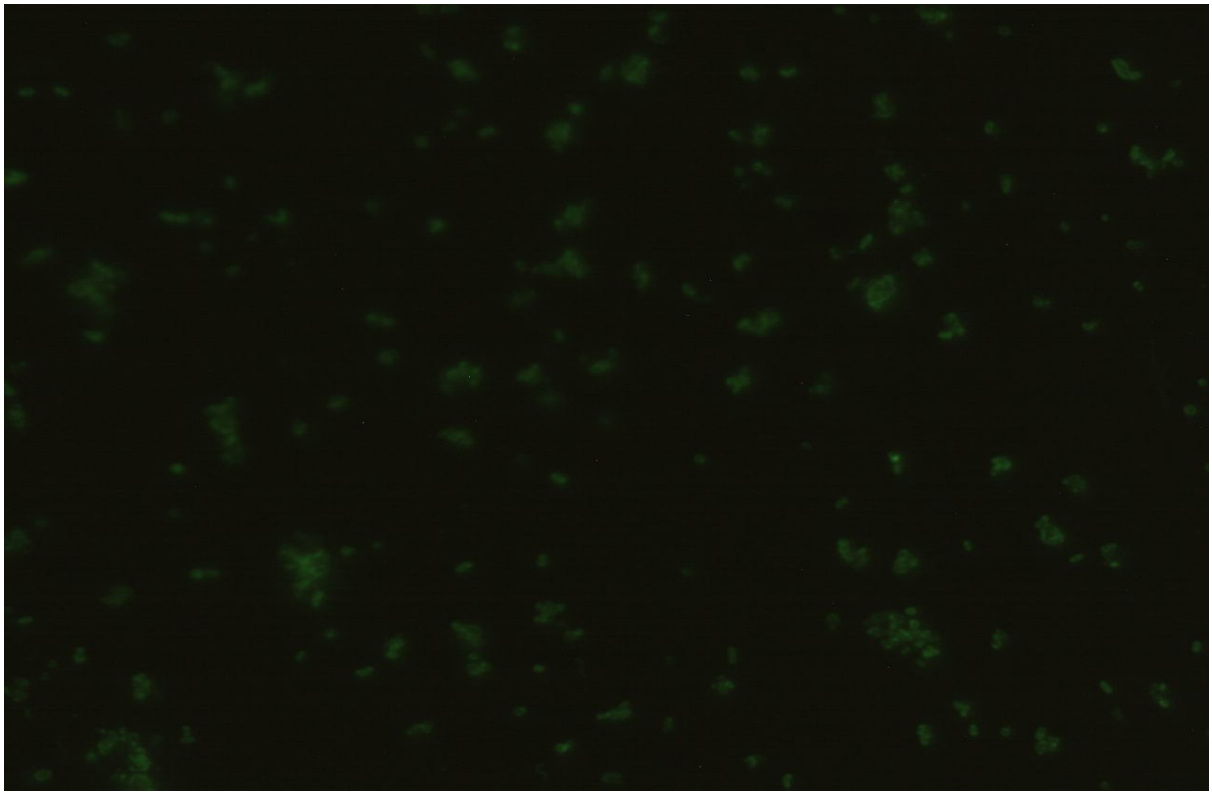


Fig (**10**) Immunofluorescence showing +++

These Image shows (highly infection) of the fluorescence pattern appears in parts circular or granular under fluorescence microscope .

Antibodies against *Helicobacter Pylori* IgG are present .

+ve

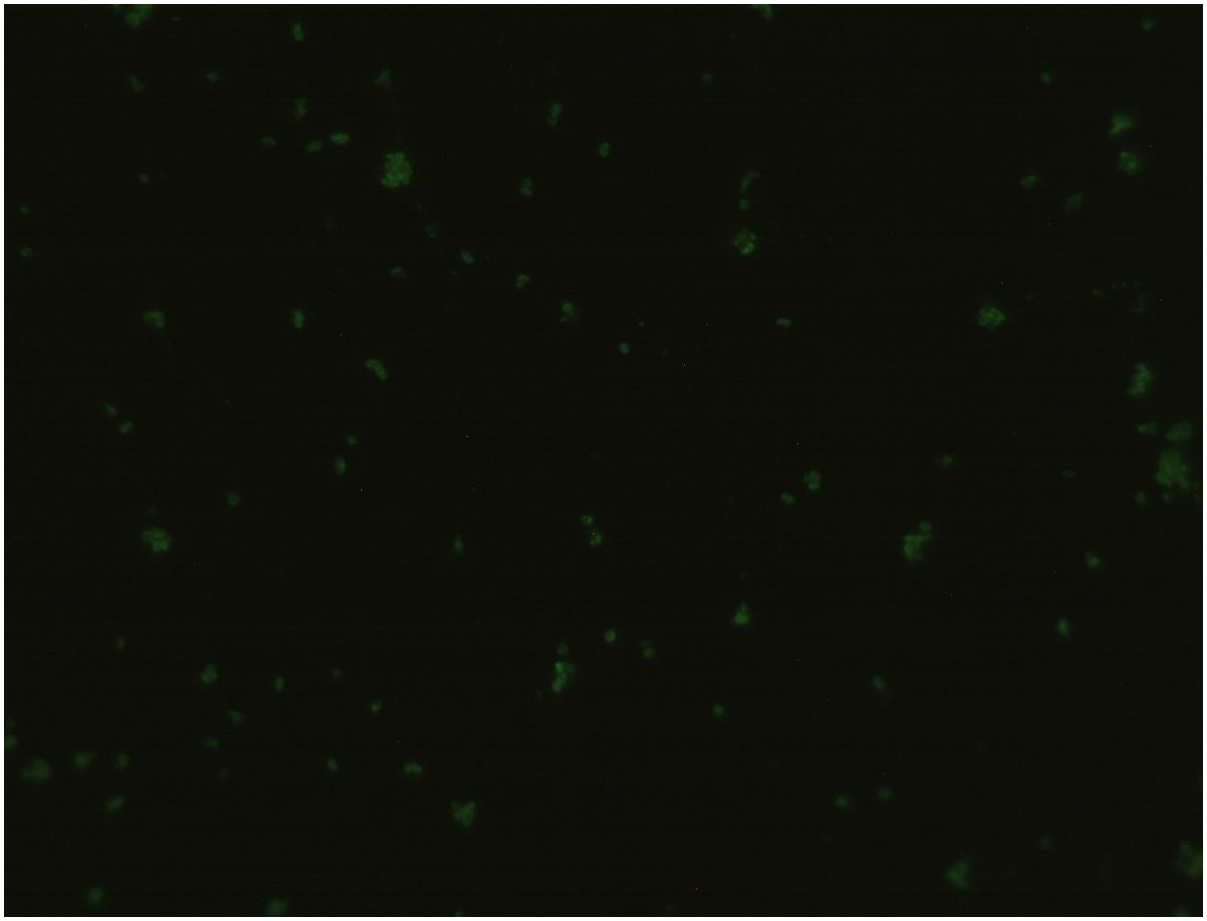


Fig (11) Immunofluorescence showing +ve

These Image shows (positive control) of the fluorescence pattern appears in parts circular or granular under fluorescence microscope .

Antibodies against *Helicobacter Pylori* IgG are present .

-ve

Fig (12) Immunofluorescence showing -ve

These Image shows (negative control) no fluorescence pattern appears in parts under fluorescence microscope .

No Antibodies against *Helicobacter Pylori* IgG are present .

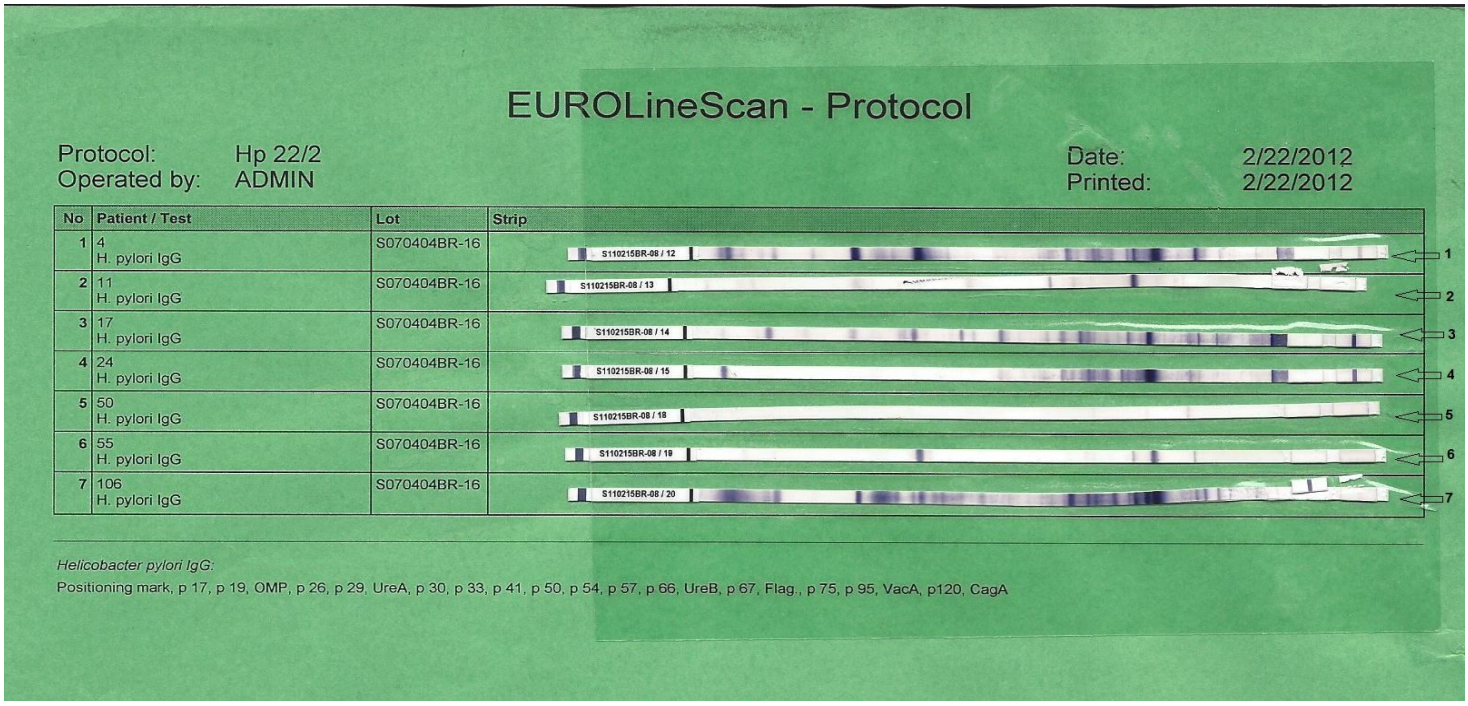


Fig (13) EUROLINE WB Scan anti *Helicobacter pylori* IgG

1.*Helicobacter pylori* IgG positioning strong mark p120,p57,p54,p41,p26 and weak mark p 67

(*Helicobacter pylori* IgG positive)

2.*Helicobacter pylori* IgG positioning strong mark p120,p54,p30 and weak mark p 57

(*Helicobacter pylori* IgG positive)

3.*Helicobacter pylori* IgG positioning strong mark p120,p54,p30,p26,p41,p57 and weak mark p 17

(*Helicobacter pylori* IgG positive)

4.*Helicobacter pylori* IgG positioning strong mark p120,p54, p41, p33 and weak mark p57, p26

(*Helicobacter pylori* IgG positive)

5.*Helicobacter pylori* IgG no mark in protein strips . (Helicobacter pylori IgG negative)

6.*Helicobacter pylori* IgG positioning strong mark p120,p54, p41, p26 .

(*Helicobacter pylori* IgG positive)

7.*Helicobacter pylori* IgG positioning strong mark p57,p54, p41, p26 .p30 and weak mark p120,p29.

(*Helicobacter pylori* IgG positive)

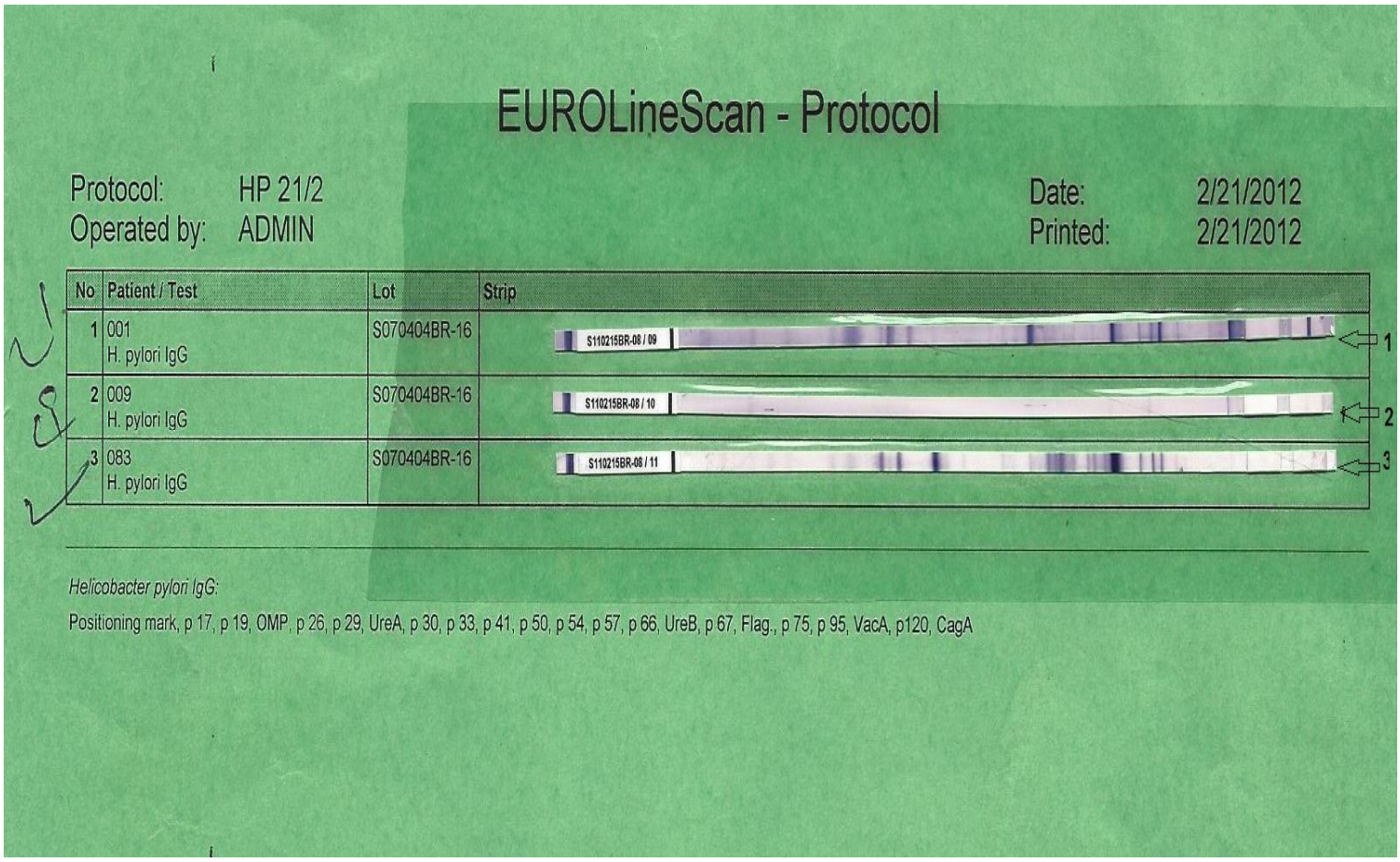


Fig (14) EUROLINE WB Scan anti *Helicobacter pylori* IgG

1.*Helicobacter pylori* IgG positioning strong mark p57,p54, p50, p26 .p120 and weak mark p67.

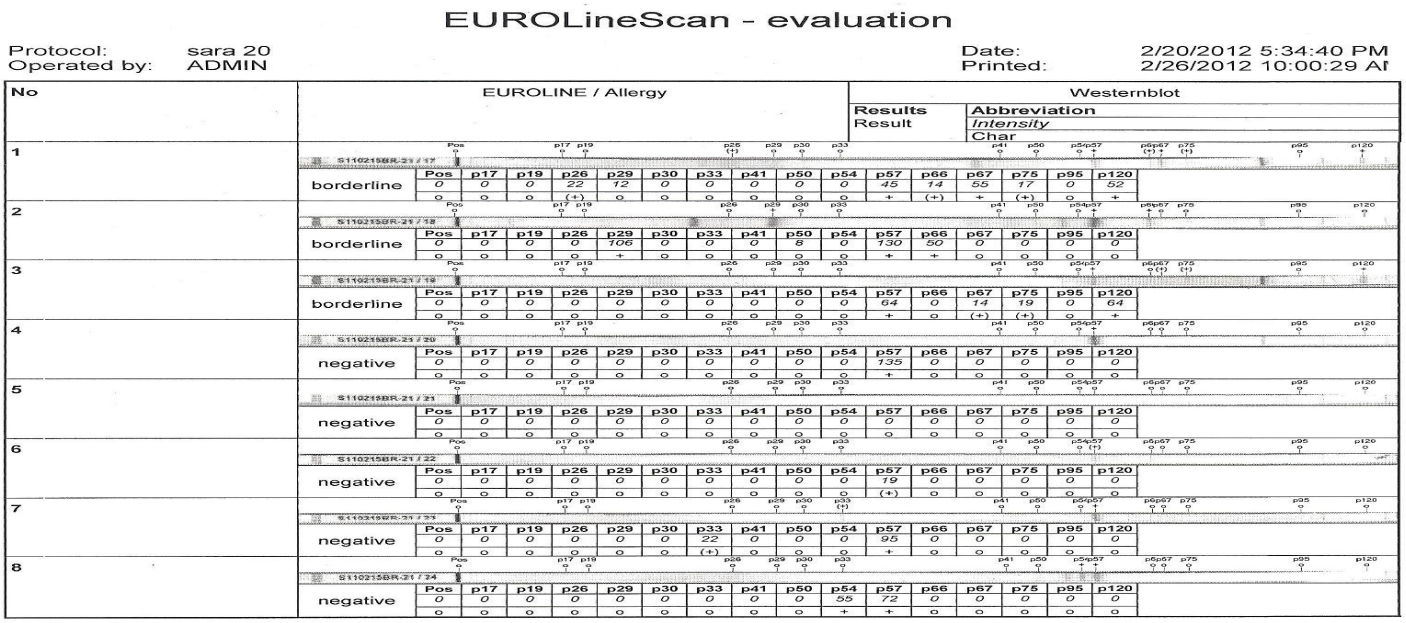
(*Helicobacter pylori* IgG positive)

2.*Helicobacter pylori* IgG no mark in protein strips .

(*Helicobacter pylori* IgG negative)

3.*Helicobacter pylori* IgG positioning strong mark p57,p54, p41, p26 .p120

(*Helicobacter pylori* IgG positive).



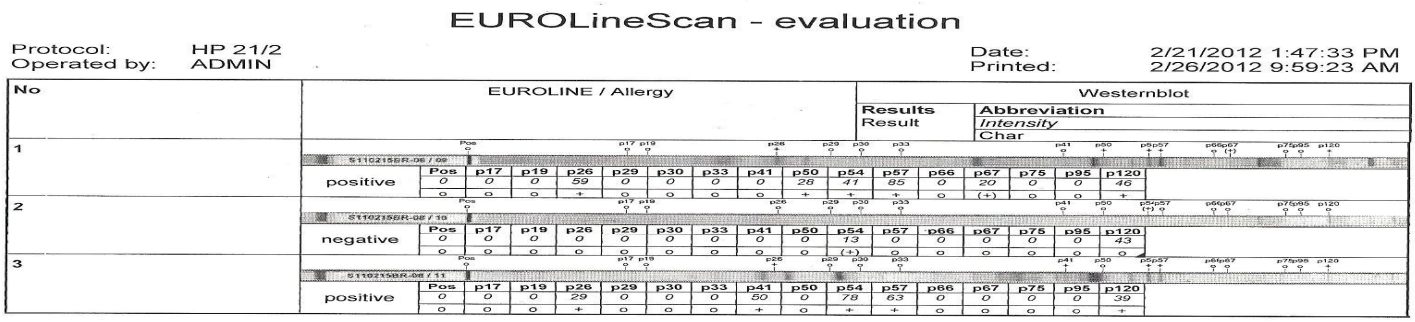
EUROLineScan - evaluation

Protocol: sara 20
Operated by: ADMIN

Date: 2/20/2012 5:34:40 PM
Printed: 2/26/2012 10:00:29 AM

No	EUROLINE / Allergy	Westernblot
		Results / Result: Abbreviation / Intensity / Char
1	S110215BR-21 / 17 — borderline	Pos 0 · p17 0 · p19 0 · p26 22 · p29 12 · p30 0 · p33 0 · p41 0 · p50 0 · p54 0 · p57 45 · p66 14 · p67 55 · p75 17 · p95 0 · p120 52
		o o o (+) o o o o o + (+) + (+) o +
2	S110215BR-21 / 18 — borderline	Pos 0 · p17 0 · p19 0 · p26 0 · p29 106 · p30 0 · p33 0 · p41 0 · p50 0 · p54 8 · p57 130 · p66 50 · p67 0 · p75 0 · p95 0 · p120 0
		o o o o + o o o o o + + o o o o
3	S110215BR-21 / 19 — borderline	Pos 0 · p17 0 · p19 0 · p26 0 · p29 0 · p30 0 · p33 0 · p41 0 · p50 0 · p54 0 · p57 64 · p66 0 · p67 14 · p75 19 · p95 0 · p120 64
		o o o o o o o o o o + o (+) (+) o +
4	S110215BR-21 / 20 — negative	Pos 0 · p17 0 · p19 0 · p26 0 · p29 0 · p30 0 · p33 0 · p41 0 · p50 0 · p54 0 · p57 135 · p66 0 · p67 0 · p75 0 · p95 0 · p120 0
		o o o o o o o o o o + o o o o o
5	S110215BR-21 / 21 — negative	Pos 0 · p17 0 · p19 0 · p26 0 · p29 0 · p30 0 · p33 0 · p41 0 · p50 0 · p54 0 · p57 0 · p66 0 · p67 0 · p75 0 · p95 0 · p120 0
		o o o o o o o o o o o o o o o o
6	S110215BR-21 / 22 — negative	Pos 0 · p17 0 · p19 0 · p26 0 · p29 0 · p30 0 · p33 0 · p41 0 · p50 0 · p54 0 · p57 19 · p66 0 · p67 0 · p75 0 · p95 0 · p120 0
		o o o o o o o o o o (+) o o o o o
7	S110215BR-21 / 23 — negative	Pos 0 · p17 0 · p19 0 · p26 0 · p29 0 · p30 0 · p33 22 · p41 0 · p50 0 · p54 0 · p57 95 · p66 0 · p67 0 · p75 0 · p95 0 · p120 0
		o o o o o o (+) o o o + o o o o o
8	S110215BR-21 / 24 — negative	Pos 0 · p17 0 · p19 0 · p26 0 · p29 0 · p30 0 · p33 0 · p41 0 · p50 0 · p54 0 · p57 55 · p66 72 · p67 0 · p75 0 · p95 0 · p120 0
		o o o o o o o o o + + o o o o o

Fig (15) EUROLINE WB Scan a Evaluation anti *Helicobacter pylori* IgG



EUROLineScan - evaluation

Protocol: HP 21/2
Operated by: ADMIN

Date: 2/21/2012 1:47:33 PM
Printed: 2/26/2012 9:59:23 AM

No	EUROLINE / Allergy	Westernblot
		Results / Result: Abbreviation / *Intensity* / Char
1	S1102155R-06 / 09 — positive	Pos 0 o; p17 0 o; p19 0 o; p26 59 +; p29 0 o; p30 0 o; p33 0 o; p41 0 o; p50 28 +; p54 41 +; p57 85 +; p66 0 o; p67 20 (+); p75 0 o; p95 0 o; p120 46 +
2	S1102155R-0E / 10 — negative	Pos 0 o; p17 0 o; p19 0 o; p26 0 o; p29 0 o; p30 0 o; p33 0 o; p41 0 o; p50 0 o; p54 13 (+); p57 0 o; p66 0 o; p67 0 o; p75 0 o; p95 0 o; p120 43 o
3	S1102155R-0E / 11 — positive	Pos 0 o; p17 0 o; p19 0 o; p26 29 +; p29 0 o; p30 0 o; p33 0 o; p41 50 +; p50 0 o; p54 78 +; p57 63 +; p66 0 o; p67 0 o; p75 0 o; p95 0 o; p120 39 +

Fig (16) EUROLINE WB Scan a Evaluation anti *Helicobacter pylori* IgG

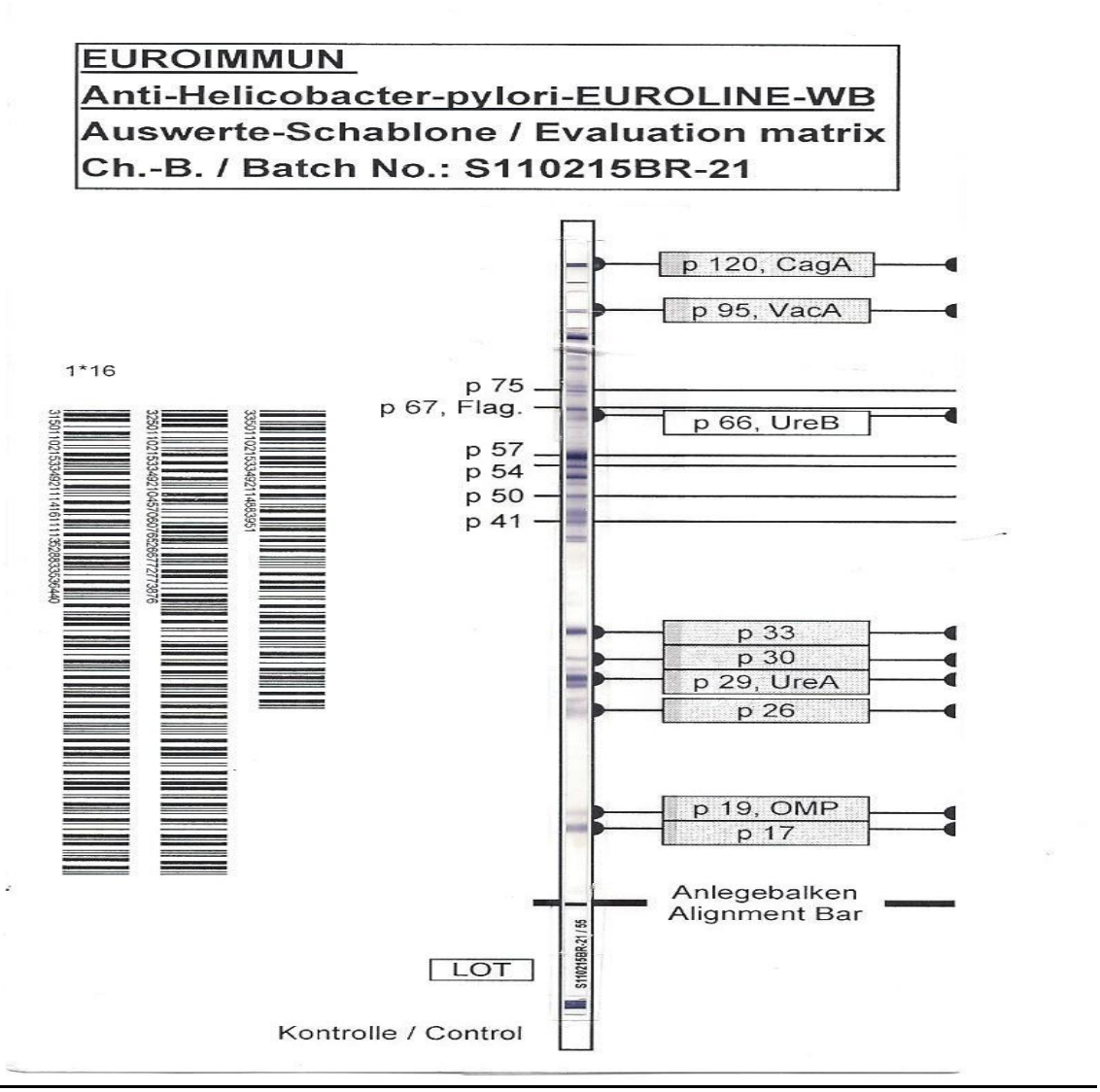


Fig (17) EUROLINE WB Scan a anti *Helicobacter pylori* IgG (Positive control +ve)

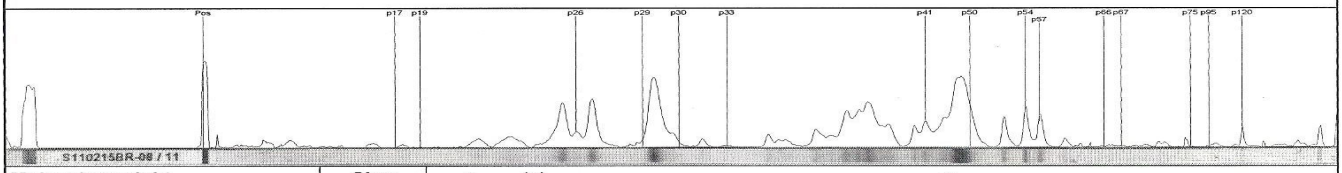
RAYAN Specialized Laboratory

Khartoum - Alsied A . Alrahman Streat \ Alqaser Streat

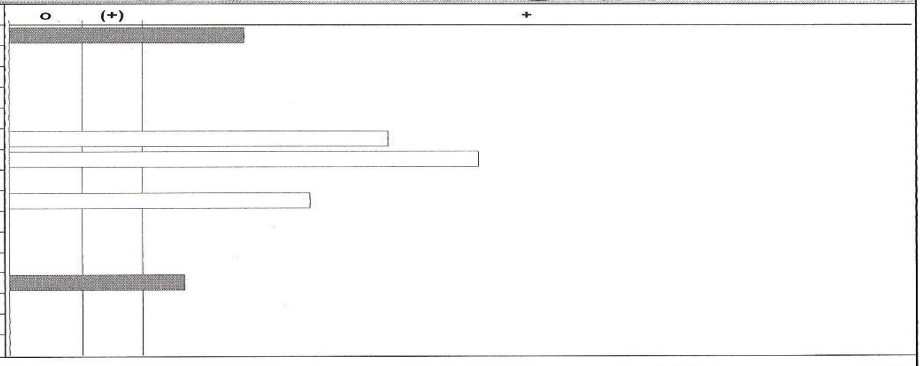
Patients IDs:	083	**Test:**	Helicobacter pylori IgG
Name:	083	**Lab number:**	083
Date of receipt:	2/21/2012	**Number:**	3
Results from:	2/26/2012		

EUROIMMUN Medizinische Labordiagnostika AG

Automatic evaluation of test strips using the EUROLineScan software



Molecular weight	Char
p120, CagA (p120)	+
p 95, VacA (p95)	o
p 75 (p75)	o
p 67, Flag. (p67)	o
p 66, UreB (p66)	o
p 57 (p57)	+
p 54 (p54)	+
p 50 (p50)	o
p 41 (p41)	+
p 33 (p33)	o
p 30 (p30)	o
p 29, UreA (p29)	o
p 26 (p26)	+
p 19, OMP (p19)	o
p 17 (p17)	o
Positioning mark (Pos)	o



Class	Explanation
o	No staining
(+)	Weak staining
+	Strong staining

Test	Result
Helicobacter pylori IgG	positive

Signature: _____

Fig (18) Automatic Evaluation of test strip EUROLINE WB Scan a anti *Helicobacter pylori* IgG (Positive +ve).

RAYAN Specialized Laboratory

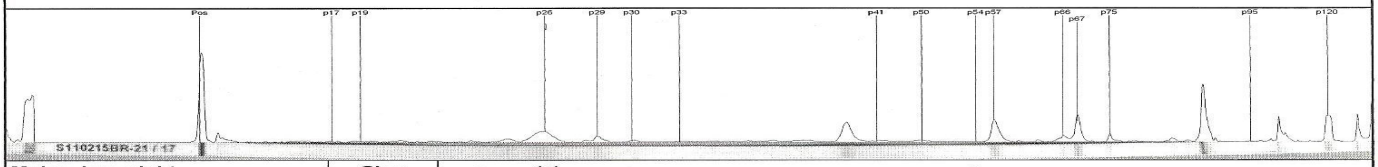
Khartoum - Alsied A . Alrahman Streat \ Alqaser Streat

Patients IDs: P106
Date of receipt: 2/20/2012
Results from: 2/26/2012

Test: Helicobacter pylori IgG
Number: 1

EUROIMMUN Medizinische Labordiagnostika AG

Automatic evaluation of test strips using the EUROLineScan software



Molecular weight	Char
p120, CagA (p120)	+
p 95, VacA (p95)	o
p 75 (p75)	(+)
p 67, Flag. (p67)	+
p 66, UreB (p66)	(+)
p 57 (p57)	+
p 54 (p54)	o
p 50 (p50)	o
p 41 (p41)	o
p 33 (p33)	o
p 30 (p30)	o
p 29, UreA (p29)	o
p 26 (p26)	(+)
p 19, OMP (p19)	o
p 17 (p17)	o
Positioning mark (Pos)	o

Class	Explanation
o	No staining
(+)	Weak staining
+	Strong staining

Test	Result
Helicobacter pylori IgG	borderline

Signature: _____

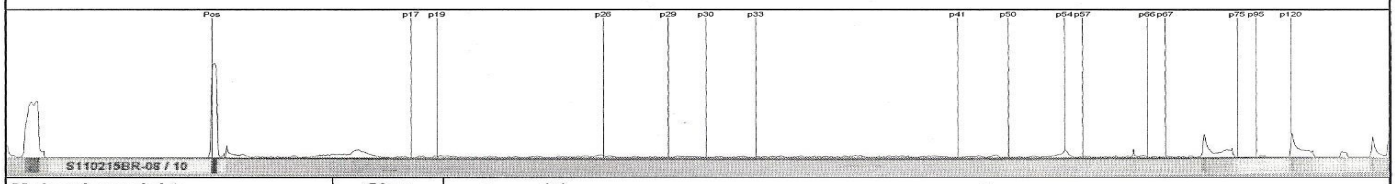
Fig (19) :Automatic Evaluation of test strip EUROLINE WB Scan a anti *Helicobacter pylori* IgG (borderline? ve)

RAYAN Specialized Laboratory — **Khartoum - Alsied A . Alrahman Streat \ Alqaser Streat**

Patients IDs:	009	**Test:**	Helicobacter pylori IgG
Name:	009	**Lab number:**	009
Date of receipt:	2/21/2012	**Number:**	2
Results from:	2/26/2012		

EUROIMMUN Medizinische Labordiagnostika AG

Automatic evaluation of test strips using the EUROLineScan software



Molecular weight	Char	o	(+)	+
p120, CagA (p120)	o			
p 95, VacA (p95)	o			
p 75 (p75)	o			
p 67, Flag. (p67)	o			
p 66, UreB (p66)	o			
p 57 (p57)	o			
p 54 (p54)	(+)			
p 50 (p50)	o			
p 41 (p41)	o			
p 33 (p33)	o			
p 30 (p30)	o			
p 29, UreA (p29)	o			
p 26 (p26)	o			
p 19, OMP (p19)	o			
p 17 (p17)	o			
Positioning mark (Pos)	o			

Class	Explanation
o	No staining
(+)	Weak staining
+	Strong staining

Test	Result
Helicobacter pylori IgG	negative

Signature: _____

Fig (20) : Automatic Evaluation of test strip EUROLINE WB Scan a anti Helicobacter pylori IgG (negative -ve)

EUROIMMUN Medizinische Labordiagnostika AG

(EV_0010_F_DEUK_A04) EV_0010_F_DEUK_A04_13351000-0092-03.doc

ELISA Incubation

			antigen-coated wells	
1.	**Pipettieren:** ***Pipette:***	*100 µl per well*	*calibrators, controls, samples*	
	Inkubieren: ***Incubate:***	*30 min at room temperature (18°C to 25°C)*		
	Waschen: ***Wash:***	*300 µl (man.)/450 µl (aut.) per well* reaction time: 30-60 s per washing cycle		3x
2.	**Pipettieren:** **Pipette:**	100 µl per well	*enzyme conjugate*	
	Inkubieren: ***Incubate:***	*30 min at room temperature (18°C to 25°C)*		
	Waschen: ***Wash:***	*300 µl (man.)/450 µl (aut.) per well* *reaction time: 30-60 s per washing cycle*		3x
3.	**Pipettieren:** **Pipette:**	100 µl per well	*chromogen/substrat*	
	Inkubieren: ***Incubate:***	*15 min at room temperature (18°C- 25°C)*		
	Pipettieren: **Pipette:**	100 µl per well	*stop solution*	
	Auswerten: ***Evaluate:***	*photometric measurement (450 nm)*		

Fig (21) : Method of ELISA.

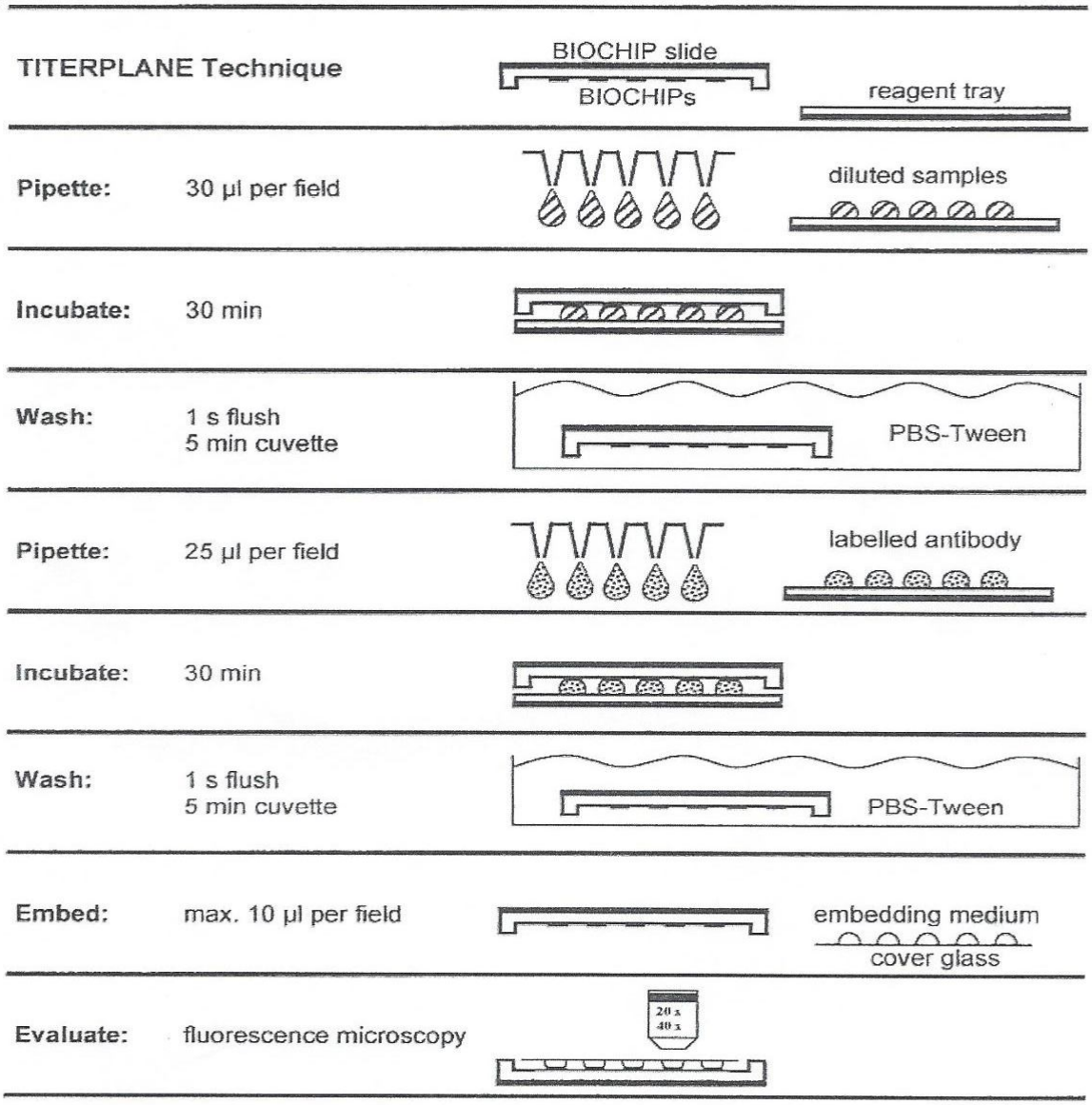


Fig (22) : Method of Indirect immunoflurorescence.

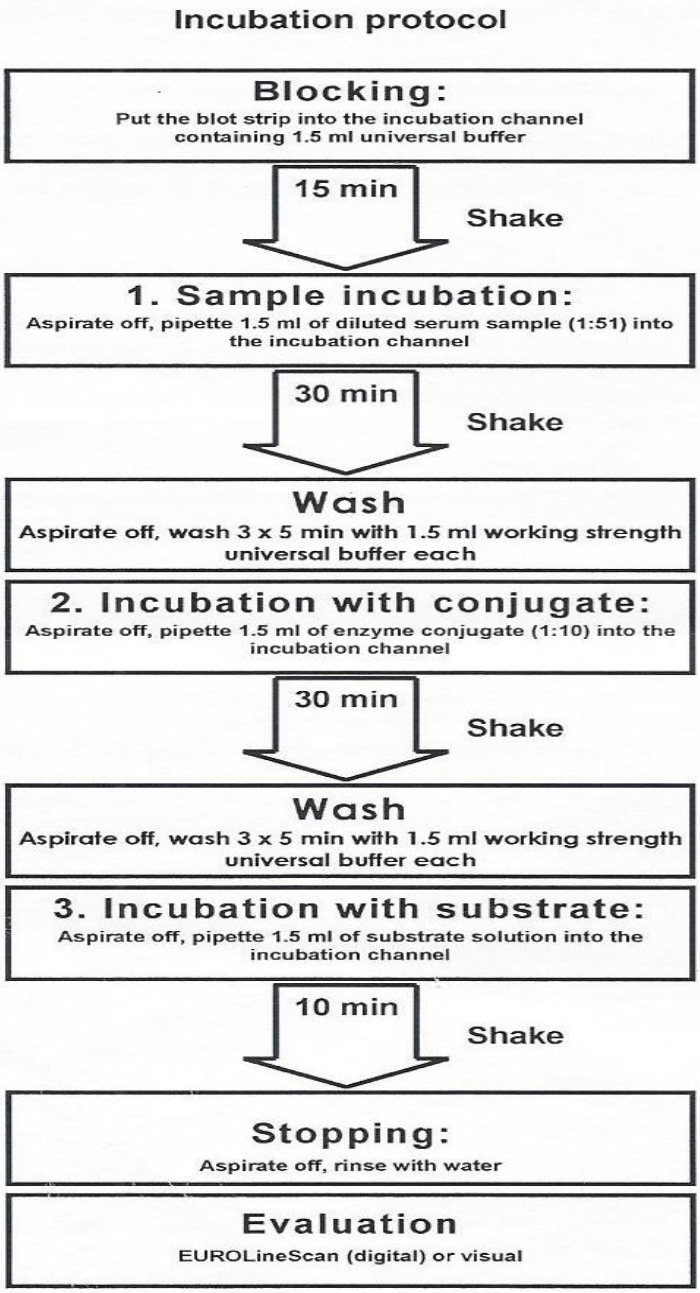
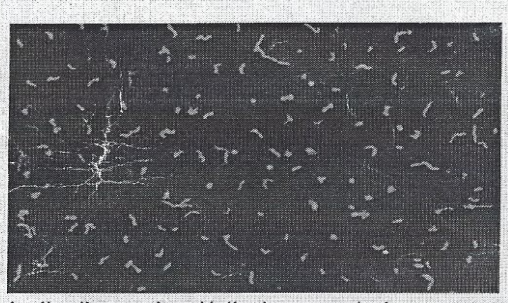


Fig (23) EUROLINE WB protocol anti *Helicobacter pylori* igG

EUROIMMUN Products for Infectious Serology

Antibodies against Helicobacter pylori

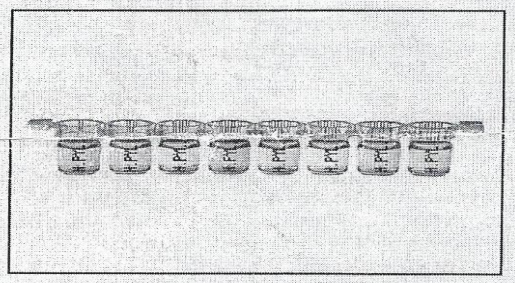


Antibodies against Helicobacter pylori.

Indirect Immunofluorescence Test: Anti-Helicobacter pylori

- Sensitive screening test for the detection of antibodies against Helicobacter pylori.
- Indications: gastritis, ulcus ventriculi et duodeni. Late consequences: MALT lymphomas and adenocarcinomas.
- Initial dilution 1:10 (IgM), 1:100 (IgG), 1:32 (IgA).
- If antibodies against Helicobacter pylori are present, a distinct fluorescence of the bacteria in the smear is obtained.
- A positive IgA result correlates well with the activity of a gastritis. An increased IgG antibody titer is considered to be a marker for chronic infections. A significant drop in the IgG antibody titer about 6 months after therapy is a sign of success.
- The BIOCHIP can be supplemented as required with further substrates, e.g. other infectious agents or tissue sections of primate stomach.

Order No.	Formats
FI 2080-#### A, G or M	page 133

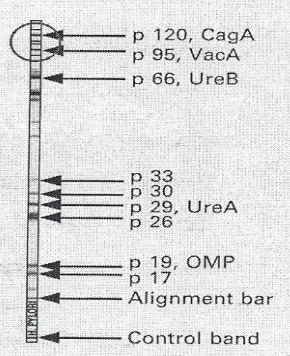


Incubated ELISA Anti-Helicobacter pylori.

Microplate ELISA: Anti-Helicobacter pylori

- Sensitive screening test for the detection of antibodies against Helicobacter pylori.
- Indications: gastritis, ulcus ventriculi et duodeni. Late consequences: MALT lymphomas and adenocarcinomas.
- Serum dilution 1:101; conjugate class anti-IgA or anti-IgG, POD-labelled.
- Antibodies against Helicobacter pylori antigens can be determined quantitatively in RU/ml.
- 1-point calibration, semi-quantitative (IgA) or 3-point calibration, quantitative (IgG). Identical incubation conditions and times: both tests can be combined without difficulty on one and the same microplate.
- Native antigens.

Order No.	Formats
EI 2080-9601 A or G	page 93



Incubated EUROLINE-WB Anti-Helicobacter pylori.

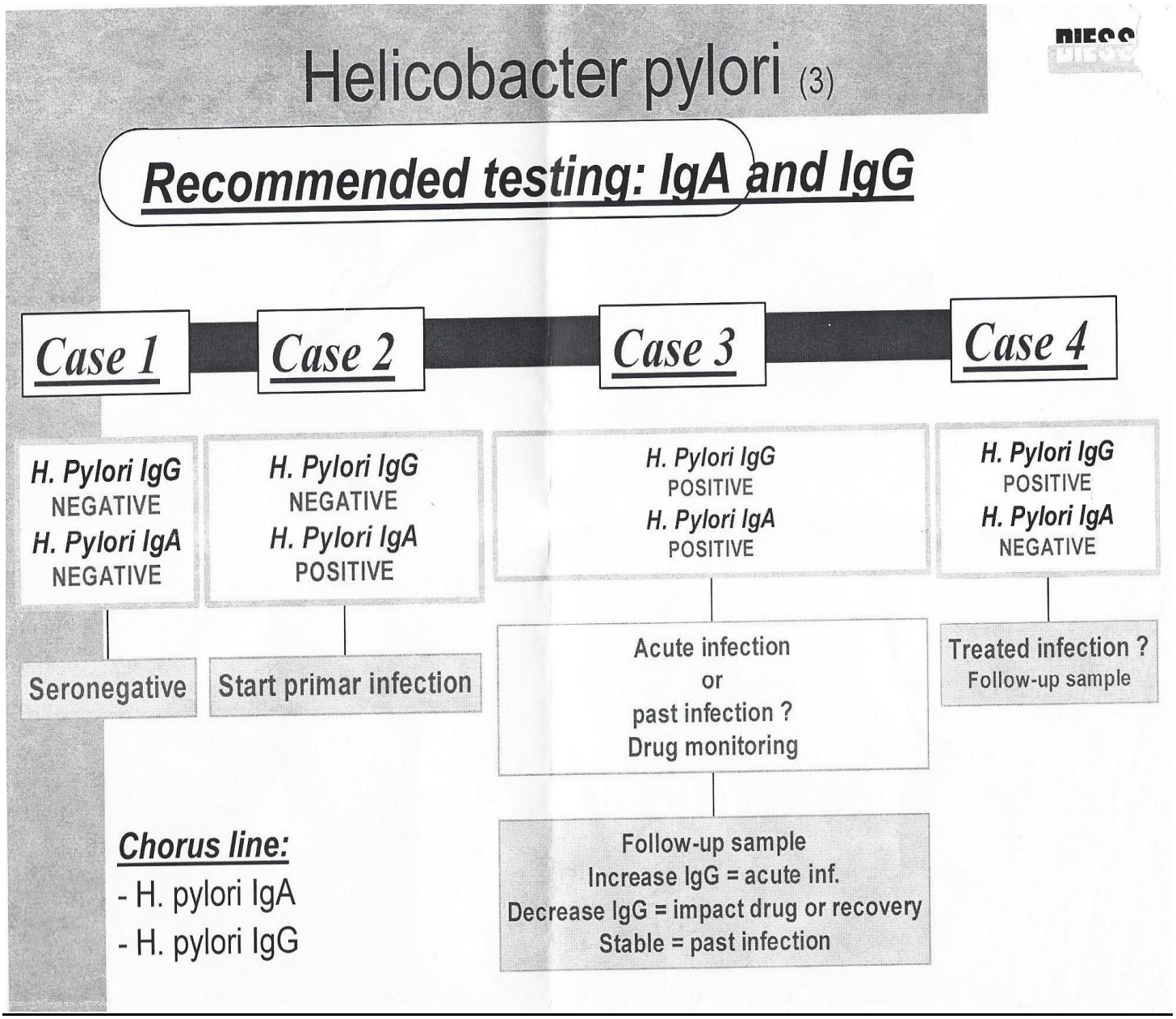
EUROLINE-WB: Anti-Helicobacter pylori

- Specific confirmatory test for the detection of antibodies against Helicobacter pylori.
- Indications: gastritis, ulcus ventriculi et duodeni. Late consequences: MALT lymphomas and adenocarcinomas.
- Serum dilution 1:50; conjugate class anti-IgA or anti-IgG, AP-labelled.
- EUROLINE-WB is a combination of westernblot and line blot techniques. An SDS extract of a Helicobacter pylori strain is electrophoretically separated according to molecular mass and transferred onto a nitrocellulose membrane (westernblot). Two membrane chips coated with highly purified recombinant CagA and VacA are subsequently applied to the westernblot strips.
- Bands from all specific antigens are included and clearly separated.
- Test strips can be automatically incubated and evaluated using the systems EUROBlotMaster und EUROLineScan (see page 27).

Order No.	Formats
DY 2080-1601-1 A or G	page 86

Fig (24) : Different between ELISA ,Indirect immunoflurorescence and Euroline – WB .

S



Fig(25) : Different between IgG and IgA antibodies against *Helicobacter pylori*.

-How to start a test run

Blot Master

1. Switch on device (back left hard side)

Main menu: Lists of assays	Confirm **ENTER**

2. Select test programme

Lists of assays: 01 Euro01 AAb EL30	I. Select test using **YES +** or **NO -**

3. Enter number of strips

No of strips: 00 Max: 30	- Enter number of strip using **YES** - Confirm **ENTER**

4. Load fluid

Pump A: wash buffer xx ml - Priming, + Postpone, **ENTER** Ready	I. Use **NO -** to fill tube with reagent (push key twice)

- Reagent type and volume for channel A is shown in the top line on the display.
- It is recommended to prepare **5 mL** reagent in addition for priming.

Pump B: IgA conjugate xx ml - Priming , + Postpone, **ENTER** Ready	I. Use **NO** to fill tube with reagent (twice)

*** There is a possibility to postpone the reagent insertion by using YES – key (+ postpone). The control LED is switched OFF.

The reagent requested **5 minutes** before it is application. The function is usually available for **substrate only**.

5. **After the priming of each channel, the display read**

Is waste bottle empty?	Check waste container Confirm

6. **Start assay**

Start assay? 01 Euro01 AAb EL30	Confirm **YES**

7. **Insert strip**

Step 01 – 1: Strips S 00:01 Insert strips? Complete? A 01:53	Confirm **YES**

8. **Add sample**

The EUROBlotMaster file the channels with diluent buffer.

Step 04-1: Sample incub. S 00:30 Add 15ul sample Compl.? A 01:47	Confirm **YES**

9. **Aspiration**

Step 09-3: Sample incub. S 00:30 Aspiration strips? Compl.? A 00:03	Confirm **YES**

10. **Finish test run**

Finish assay? A 00:00 Waiting time 00:05	Confirm **YES**

The incubation tray can now be removed for evaluation .Continue with the daily cleaning

11. Daily Maintenance

- After each test run, the display read:

Pumps priming: Priming all pumps?	Confirm **YES**

- Place all input tubes into a container with distilled water.

Volume per pump: 15.0 ml	Set volume to 15 ml using **YES** Confirm **ENTER**

12. Empty all tubes

Pumps priming: Priming all pumps?	Confirm **YES**

- Take out all tube ends and put them on a paper towel to avoid dropping.

Volume per pump: 10.0 m	Set volume to 10 ml using **YES** Confirm **ENTER**

13. Switched off

Main menu: List of assay	Instrument can be switched off

Appendix V

QUESTIONAIRE

- **الاسم / NAME : .....**
- **العمر / Age :.....**
- **الجنس / sex :.....**
- **الجنسية / Nationality : .....**
- **المنطقة / City: .....**
- **التلفون / Phone: .....**

Please …answer about the questions لطفا ..اجب على الاسئله التاليه

1/Are you infected with *H .Pylor*i ? هل يوجد اي اصابه مسبقه بجرثومه المعده؟

.....

2/Do you have any symptoms? هل يوجد اي اعراض للاصابه بجرثومه المعده ؟

.....

3/If there...how long? وفي حال الاصابه ...متى ظهرت؟

.....

4/Do you have any GIT symptoms like stomach acidity heart burn pain in theb stomach or dark stool?

هل يوجد اي اعراض لمشاكل الجهاز الهضمي مثل حموضه المعده وحرقان في فم المعده والام في المعده او لون البراز اسود ؟

.....

5/Any one at home has these symptoms ?

هل يوجد احد من المخالطين لك في المنزل يعاني من اعراض في المعده؟

.....

6/Any one at home received treatment for stomach problem ?

هل يوجد احد من المخالطين لك في المنزل يتناول ايه علاجات لمشاكل المعده ؟

.....

7/What is a last result ?When? ماهي نتيجه اخر تحليل للجرثومه...ومتى؟

.....

8/Have you taken any mediation?

هل تناول المريض اي علاج لجرثومه المعده ...او اي التهاب في الجهاز الهضمي ؟

.....

9/Are you ok after treatment ? ماهي حاله المريض حاليا؟

.....

10/Did you have any chronic disease ? هل يعاني المريض من اي امراض مزمنه ؟

.....

بهذا اوكد اني فهمت الهدف من الدراسه وماهو مطلوب مني ,لقد اتيحت لي فرصه كافيه لمراجعه المعلومات المتوفره,مقدمه الاسئله اللازمه حولها وتلقيت اجابات مرضيه ,واعلم ان مشاركتي هي امر اختياري وان بوسعي التخلي في اي وقت دون ان يؤثر ذلك على حقوقي القانونيه او ماتلقاه من رعايه صحيه.

واخيرا , اوافق على الاسهام في البحث المذكور اعلاه .

وشكرا

I confirm that I understood the purpose of the study and what is required from me . I had the opportunity to consider the information , ask questions and had these answered satisfactorily . I understand that my participation is voluntary and that I am free to withdraw at any time , without my medical care or legal right being affected .

I gree to take part in the above research study .

Name of subject: .....:اسم المشارك

Name of guardian: .....:اسم ولى الامر

Signature:.....:التوقيع