PERFORMANCE OF ONE-STEP SPANBIO™ RAPID TEST IN DETECTION OF *GIARDIA LAMBLIA* IN BUSIA COUNTY REFERRAL HOSPITAL, WESTERN KENYA

BY

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DECLARATION

Declaration by the student

I declare that this is my original thesis and that it has not been presented to any other institution for a Degree or any other award.

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Approval by the Supervisors:

This research has been carried out under our supervision and has been submitted to Maseno University School of Graduate Studies for examination by my approval as the candidate's supervisor.

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DEDICATION

To my family for their moral support, and perseverance during my long absence from home. Also the thesis is dedicated to my parents, brothers and sisters for their prayers and motivation. Also to my friends and classmates.

ABSTRACT

The protozoan flagellate parasite *Giardia lamblia* is the aetiologic agent for giardiasis, an infectious disease with significant burden in developing countries. Currently, clinical signs and symptoms accompanied by microscopy are used to diagnose giardiasis. However, microscopic stool analysis is limited by low sensitivity, labor intensive and high turn-around time. Moreover, polymerase chain reaction (PCR) has been applied in the detection of *G.lamblia*, but its cost and technical skills have compromised its clinical utilization. One-step rapid diagnostic tests (RDT) such as the SPANBIO™ have been developed for infection screening purposes due to high turn-around time and use in point-of-care testing. SPANBIO™ RDT is based on the principle of immuno-chromography. However, its utility in detection of giardiasis in Kenya has not been evaluated. This was cross-sectional health facility-based study that sought to evaluate the diagnostic performance of SPANBIO™ RDT in detection of *Giardia lamblia* with microscopy as the gold standard and PCR as a reference standard in a clinical setting. The specific objective were; To determine the specificity of SPANBIO™ RDT against microscopy and PCR in detecting *G. lamblia* in diarrheal patients, to determine the sensitivity of SPANBIO™ RDT against microscopy and PCR in detecting *G. lamblia* in diarrheal patients, to identify factors influencing SPANBIO™ RDT test performance in diarrheal patients. Data collection of one hundred and forty-seven stool specimens from microscopy confirmed *G. lamblia* infected =78 and uninfected =69 individuals, were documented including demographic and clinical information. The patients collected about 10gms of sample (after instructions) from which only 2gms (peas size) were processed and examined macroscopically and microscopically by the direct stool analysis procedure. Subsequently, the stools specimens were analyzed using the SPANBIO™ one-step RDT according to the manufacturer's protocols. Total genomic DNA extraction was done on 2 gms of stool and PCR was performed by amplification of GDH (5′-TCAACGTCAACCGCTTCCT-3′) gene. Relative to the gold standard SPANBIO™ RDT illustrated a sensitivity of 66.7% (95% CI; 55.1-76.9%) and specificity of 98.6% (95% CI; 92.3-100%) with positive predictive value and negative predictive value of 98.1% (95% CI; 88.1%-99.7%) and 72.3% (95% CI; 65.6-78.1%) respectively; The test agreement between the SPANBIO™ and microscopy was high, and is indicated by Cohen′s kappa coefficient = 0.6388; $P<0.0001$). When compared to PCR, RDT had sensitivity and specificity of 78.2 %, (95% CI; 67.4-86.8%) and 89.7% (95% CI; 80.2-95.8%) respectively and a positive predictive value and negative predictive value of 89.7 % (95% CI; 81.0-94.7%) and 78.5% (95% CI; 70.4-84.8%), respectively. The test agreement between the SPANBIO™ and PCR was high, and is indicated by Cohen′s kappa coefficient = 0.6750; $P<0.0001$). Analyses to determine factors influencing SPANBIO™ RDT performance indicated that mucus, 2.982(95% CI; 0.089-0.440) and fecal pus 2.318(95% CI; 0.035-0.439) in stool affected its performance, *G. lamblia* was commonly found in semi-formed and loose stool. Therefore, there should also be a way of improving sample quality for giardiasis diagnosis. In conclusion the results obtained have shown that although mucus and fecal pus influence test performance, SPANBIO™ RDT is equally can be used in the diagnosis of *G. lamblia*. This study has significance as it has shown that there is an alternative technique that can produce timely, reliable and non-laborious results in the diagnosis of *G. lamblia*, which can be used in areas without microscopic capabilities.

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LIST OF ACRONYMS AND ABBREVIATIONS

AIDS	Acquired Immuno-deficiency Syndrome
CD	Cluster of differentiation
CDC	Center for Disease Control
CLSI	Clinical and Laboratory Standards Institute
DFA	Direct Fluorecent Antibody
DNA	Deoxyribonucleic acid
EIA	Enzyme Immunoassays
ELISA	Enzyme-linked immuno-sobent assay
FEA	Formal-ethyl-acetate
GDH	Glutamate dehydrogenase
IBS	Irritable Bowel Syndrome
IIF	Indirect Immuno-fluorence
IFA	Immunofluorescence assay
IL	Interleukin
KIHBS	Kenya Integrated Household Budget Survey
KHIS	Kenya Health Information System
KM	Kilometers
MOH	Ministry of Health
MWM	Men having sex with men
NHS-UK	National Health Services, United Kingdom
NPV	Negative Predictive Value
SPSS	Statistical Package for the Social Sciences
TPI	Triose Phosphate Isomerase
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
RBC	Red blood cells
RDT	Rapid Diagnostic Test
WBC	White blood cells
WHO	World Health Organization

TAT Turnaround Time

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CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Giardiasis (popularly known as beaver fever) is a zoonotic parasitic disease caused by the flagellate protozoan *Giardia lamblia* (also sometimes called *Giardia intestinalis* or *Giardia duodenalis*). The infection occurs in many animals including beavers, cows, rodents, and sheep. Animals are believed to play a key role in maintaining the infections in environment (Esch and Petersen, 2013). *Giardia lamblia* is the most prevalent intestinal protozoan flagellate of humans worldwide (Hooshyar *et al.,* 2018). In 2013, about 280 million people worldwide had symptomatic giardiasis (Esch and Petersen, 2013), and 500,000 new cases are reported annually. The prevalence of the disease varies from 2% - 5% in developed to 20% - 30% in developing countries (Pereira, 2007). In Sub-Saharan Africa the prevalence of diarrhea caused by *G. lamblia* is between 1.7 – 41.7% (Squire and Ryan, 2017). In Kenya, mortality in children less than five years is due to diarrhea in which water related diseases occupy a high proportion (Onyango and Ang'ienda, 2010). Prevalence of diarrheal cases at Busia county being 5.9% in 2015 (KHIS, 2015). In western Kenya, Busia included, the prevalence of giardiasis is 10% (Brett *et al.,* 2012), with the prevalence of 11.1 % on symptomatic children (Gerger, 2017).

Conventionally, the detection of *Giardia lamblia* cysts or trophozoites in duodenal, fecal, tissue, environmental and/or water samples is achieved mainly by microscopic examination but it is labor-intensive and requires experience, that is, it relies on the technician's experience and three stool samples may be required to increase sensitivity up to 90% of cases (Marks and Anando, 2015). Molecular methods, like PCR have also been employed in detection and diagnosis of giardia. Although convectional PCR seems to be the most sensitive method for detection of *G. lamblia* in human stools, it does not, as yet, provide additional advantages when used as a single test, mainly because of the risk of false-positive results (Schuurman *et al.,* 2007).

The best single test for diagnosing giardiasis is antigen testing of the stool (Marks and Anand, 2015). For antigen testing, a small sample of stool is tested for the presence of giardial proteins. The antigen test will identify more than 90% of people infected with giardia (Marks and Anand, 2015). There are several immunological techniques that employs antigen and antibody reaction as their core working principle, with varied sensitivities and specificities

(Bossche *et al.,*2016). These test can be have several steps like in the case of Enzyme Linked Immuno-sorbent assay(ELISA), Indirect Immuno-Fluorescent(IIF) assay, Direct Fluorescent Antibody(DFA) and RIDASCREEN, and those that have few steps like rapid immunoassays (Abdul-Mumin *et al.,* 2019) . These, rapid immunoassays, are valuable tool for routine clinical parasitology laboratory because of their speed and simplicity, especially when microscopic examinations of stools are not performed in the laboratory (Schuurman1 *et al.,* 2007). There are several RDT that have been used for example, Duo strip check, Vet check, Vet scan and RidaQuick but results may take up-to 30 minutes to be read and there is room for self-contamination. (Strand *et al.,* 2008).

Rapid diagnostic test like SPANBIO^{TM} utilizes rapid immunoassay principle where the area on the device is impregnated with antibody of the targeted antigen and on the control segment is the antigen of giardin protein. This test device is safe, that is, it has minimal chance for self- contamination and results are ready for interpretation just after 10 minutes, these is compared to other test devices that come as a test strip which has high chances self-contamination and takes up-to 30 minutes to get the results. Specificity, sensitivity and kappa value of these devices compared to the gold standard which is microscopy varies depending on the study (SPANBIO, 2015). The ability for a test to detect individual who have no disease, specificity, can be lower depending with, many factors, including, the type of study, the environment, including the climatic conditions and sample size, therefore, making specificity of these tests to have a range of between 80%- 100%, other kits have also these ranges. The ability of a test to detect persons who actually have the disease, sensitivity, can be affected by a range of substances including contamination and poor technique, ultimately reducing or increasing the positivity of a disease, this test has sensitivities of 60%-80%, however other kits have sensitivities as low as 52% (SPANBIO, 2015).

Factors such as alcohol and previous medication, stool features or characteristics such as presence of mucus and blood, and the parasite density may interfere with the overall performance of the test (Reynoso-Robles *et al.,* 2015). Currently, there is no known information or data that have evaluated a rapid diagnostic kit on a large-scale study in rural populations. Unlike other comparison study that focused on small population, this was a large-scale study, with a test device that results were read after 10 minutes. Therefore, evaluating the performance of one-step RDT in detection of *Giardia lamblia* in Busia County was of paramount importance as the findings will improve diagnosis of giardiasis, promote

rationale use of anti-protozoan drugs, and have a direct impact on the management and prevention of giardiasis and other diarrheal conditions.

1.2 Statement of the Problem

Treatment and management of giardiasis require prompt diagnosis, which should be accurate and reliable. Clinical signs and symptoms have been used, however some patients are asymptomatic and present non-specific (pathognomic) clinical manifestations of the disease. This may lead to abuse of drugs in the treatment of this diarrheal condition. Stool microscopy has been used to identify trophozoites and cyst but, because of intermittent shedding of trophozoites and cyst, it may requires multiple specimens to improve its accuracy. Although convectional PCR is specific and more sensitive, it is limited, giving false positive results due to contamination. Several serological methods have been employed, but are prone to false results due to cross-reactivity. Also these mentioned methods or technique are all laborious, with low turn-around time, that requires specialized training and expensive equipment, which heavily rely on the availability of electricity for them to function. Immuno-chromatographic methods have been employed in small scale human and animal studies. But information on wide scale utilization of the RDT, that is, specificity and sensitivity, in routine diagnosis is scanty. RDT are relatively cheap and easy to use and can be applied at the point-of-care. However, the performance; specificity, sensitivity and probable factors that could affect test performance of SPANBIO^{TM} RDT has not been tested in Kenya among patients of all age group presenting with giardiasis.

1.3 Justification of the study

Giardiasis is one of the diseases that cause mortality and morbidity, especially among children. Stool microscopy test is a routinely employed investigation for diagnosis of giardiasis in most clinical settings. Stool microscopy has remained the gold standard test in diagnosis, but its utility in diagnosis may need multiple samples for a true negative, and it has a low turnaround time (TAT). Currently, signs and symptoms are also available, as a diagnostic tool, especially in a resource limited setting, that have no microscopic capabilities, but these is unreliable, as signs and symptoms of giardiasis mimic other diseases, thus making the management of the condition difficult. Therefore, there is need for a specific, sensitive, accurate, inexpensive and easy method. Immuno-chromatographic methods like SPANBIO^{TM} RDT have been used successfully in small scale studies, with specificities of between 80%-100% and sensitivities of 60%-80%. Therefore, this RDT can provide reliable

tool that is simple and safe in the diagnosis of giardiasis. SPANBIO™ RDT takes a maximum of 10 minutes compared to other test procedures which can go up to 30 minutes, significantly reducing turn-around time (T.A.T) and workload, furthermore it comes as a single test. Which makes use of an alternative, one test procedure (SPANBIO™ RDT) to be relevant to medical practitioners in the diagnosis, management and prevention of the disease. Busia county was an ideal place to carry this research mainly because all risk factors associated with *Giardia lamblia* could be found in the County, these risk factors includes: shallow wells, many travelers, it borders Lake Victoria, prevalence of HIV is relatively high at 5.6%, and high poverty levels are high at 66.7 (%) based on KIHBS (2005/06).

1.4 Objectives

1.4.1 General Objective

To evaluate the performance of one-step rapid test in detection of *Giardia lamblia* in Busia county referral hospital western Kenya

1.4.2 Specific objective

i. To determine the specificity of SPANBIO™ against microscopy and PCR in detecting *G. lamblia* in diarrheal patients presenting with giardiasis in all age group of any gender.
ii. To determine the sensitivity of SPANBIO™ against microscopy and PCR in detecting *G. lamblia* in diarrheal patients presenting with giardiasis in all age group of any gender.
iii. To identify factors that influence SPANBIO™ test performance in diarrheal patients presenting with giardiasis in all age group of any gender.

1.5 Research Questions

i. What is the specificity of SPANBIO™ *G. lamblia* RDT kit compared to microscopy and PCR in detecting *G. lamblia* in diarrheal patients presenting with giardiasis in all age group of any gender?
ii. What is the sensitivity of SPANBIO™ *G. lamblia* RDT kit compared to microscopy and PCR in detecting *G. lamblia* in diarrheal patients presenting with giardiasis in all age group of any gender?
iii. What are the factors influencing the performance of SPANBIO™ *G. lamblia* RDT kit in diarrheal patients presenting with giardiasis in all age group of any gender?

1.6 Significance of the study

Timely and accurate diagnosis is the first step in the effective management and control of giardiasis. A diagnostic technique which is accurate, reliable, and cheap, and has a high TAT should be used in the management of giardiasis. The diagnosis of giardia has relied on microscopy technique which has limitations of high turnaround time, and low sensitivity and specificity. This has led to poor diagnosis with over-treatment or under treatment in clinical settings, leading to irrational use of anti-giardiasis medications. SPANBIO^{TM} RDT is a simple, in-expensive test and takes 10 minutes to report the results. The outcome of this study was important in making decisions on whether SPANBIO^{TM} RDT diagnostic technique offered a better outcome than microscopy and provided an alternative to the diagnosis of giardiasis, which could be applied in routine diagnosis of giardiasis in a clinical settings, especially the rural communities with limited resources.

1.7 Limitations of the study

Approaches in evaluating the performance of a rapid diagnostic kit include, pre-analytical stage, which include collection of stool sample, analytical stage, that is, testing of stool sample, which may include collection of fecal sample over a period of time because of the nature of how the organism is being shed in the stool and post analytical stage, recording the results. But the current study failed to do follow-up on participants who tested negative. Two weeks follow-up is advisable for participants who tested negative because the intermittent shading of giardia cyst that can go on for up to 2 weeks. Moreover there can be a possibility of cross-reaction as the study failed to isolate samples that are purely infected by *G.lamblia* species.

CHAPTER TWO
LITERATURE REVIEW

2.1 Introduction

Giardia intestinalis is a flagellated protozoa, formerly known as *Giardia duodenalis* or *Giardia lamblia* (Simmer *et al.,* 2017). Giardia were first discovered in 1681 by Antoine Van Leeuwenhoek, who found the parasite in his own stools. Giardia is a ubiquitous and well-known enteric parasite affecting humans and a range of domestic and wild mammal (Thompson *et al.,* 2016). For many years, *G. lamblia* was considered to be of doubtful pathogenicity. *Giardia lamblia* colonizes and reproduce in the small intestines causing diarrheal condition known as giardiasis. Increased awareness of this parasite and appreciation for its clinical significance surfaced in the early 1970s with its recognition in a large percentage of visitors to the Soviet Union who returned with symptomatic giardiasis (Thompson *et al.,* 2000). Giardiasis caused by *G. lamblia* is now recognized as a disease of travelers worldwide, particularly in the developing world (Thompson *et al.,* 2000).

2.2 Epidemiology of Giardia

Giardiasis is a disease of poor environmental sanitation and hence occurs in parts of the world where water supply is unsafe and sanitation is substandard. *Giardia lamblia* has a global distribution, and usually considered as the most common intestinal parasite of humans with some 280 million symptomatic cases annually (Machado-Moreiro, 2019). In developed countries the prevalence is at about 2% for adults and 6%-8% in children. In developing countries, prevalence is about 33% (CDC, 2015). Giardiasis is seasonal (Ismael *et al.*, 2016), and prevalence depends on age and location, for instance a study done in Egypt, reported a peak prevalence for both *Cryptosporidium* and *Giardia* during summer (drier months) with a second peak in winter for *Giardia* (Ismael *et al.,* 2016), in addition, studies in Kenya have reported high prevalence of intestinal parasites ranging between 12.6% to 54%, with children under age of 5 being most affected (Mbae *et al.,* 2013). *Giardia lamblia* prevalence is around 12.8% in Kenya (Thiong'o *et al.,* 2011). It should be noted that the quality and availability of data concerning giardia infection varies widely from country to country (Escobedo *et al.*, 2010). Giardia has been included into the WHO's 'Neglected Disease Initiative' from 2004 (Savioli *et al.*, 2006), this is due to its re-emergence in developed countries, and this, together with its prevalence in developing countries and its various characteristics, including symptom spectrum and possibility of long-term chronic illness (Escobedo *et al.,* 2010).

2.2.1 Etiology

Giardia intestinalis (synonyms *Giardia lamblia, Giardia duodenalis*) infects many mammalian hosts, including humans. *G.lamblia* causes giardiasis, a life threating condition among older adults, pregnant women, infants, and people who have compromised immune systems (Berrilli *et al.*, 2004). This parasite is diversified into eight morphologically identical genetic assemblages (A to H) or cryptic species, which infect different hosts (Feng and Xiao, 2011). The parasite assemblages are currently distinguishable only by PCR and sequencing of appropriate genes. Humans are infected with assemblages A and B, with the latter being predominant worldwide (Feng and Xiao, 2011). Three major sub-assemblages have been identified within assemblage A, i.e., AI, AII, and AIII. According to molecular epidemiological surveys, humans are primarily infected with AII parasites, whereas animals (including pets, livestock, and wildlife) are infected mostly by parasites belonging to sub-assemblages AI and AIII (Ryan and Cacciò, 2013).

Upon ingestion of the cyst, the cyst excyst or hatch to produce trophozoites, infection is generally restricted to intestinal lumen, causing decreased jejunal electrolyte water and glucose absorption, and damages to intestinal epithelium leading to malabsorption of electrolyte and fluids, resulting in osmotic diarrhea known as giardiasis (Huang *et al.*, 2006). Lectins and there cytopathic substance (metabolic end products) secreted by parasite also causes indirect damage to intestinal epithelium, (Huang *et al.*, 2006). The rapid multiplication of trophozoites and subsequent attachment process on the surface of duodenum and jejunum creates a physical barrier that damages microvilli, which interfere with nutrition absorption by villi. Small intestinal malabsorption and mal digestion results from the CD8+ lymphocyte-induced diffuse shortening of brush border microvilli. Activation of CD8+ lymphocytes occurs secondary to small intestinal barrier dysfunction, which results from heightened rates of enterocyte apoptosis and disruption of epithelial tight junctions (Cotton *et al.*, 2011). In addition, giardia infections can result in chronic gastrointestinal disorders such as post-infectious Irritable Bowel Syndrome (IBS) and symptoms may manifest at extra-intestinal sites, even though the parasite does not disseminate beyond the gastrointestinal tract (cotton *et al.*, 2011).

2.2.2 Life cycle

Giardia lamblia takes on two morphologically distinct forms during its life cycle. The replicative form is a motile pear-shaped cell that survives only in host small intestine called a

trophozoite (Despommier, *et al.,* 2019). Trophozoites swim through the intestinal mucus until they eventually adhere to the host intestinal epithelium by use of lectin present on the surface of the organism (Huang *et al.,* 2006). Adhered trophozoites then divide by binary fission, forming either more trophozoites or the non-replicative cyst stage. Cyst pass through the host large intestine and are shed in the feces. *G.lamblia* cyst are resistant to environment stressors, and can survive in the environment for weeks to months if kept moist. Cyst remain dormant until ingested by a host animal through contaminated food or water, person to person transmission due to poor hygiene in day care centers, nursing homes, and mental asylums and during sex, oral-anal and oral genital sex, and the cycle continues (Cernokova, *et al.,* 2018).

2.2.3 Clinical manifestation

Giardiasis incubation varies from 1-3 weeks, in majority of cases infection remains asymptomatic. Symptomatic infection is more common in children than adults because of their lower immunity (Leder *et al.,* 2011). When the infection is symptomatic it can be acute giardiasis infection or chronic giardiasis infection, in acute, the patient may present with acute watery diarrhea, abdominal cramp, bloating and flatulence signs and symptoms. Occasionally nausea, vomiting, fever, rashes or constipation in some patients may be noticeable, pus, blood and mucus are not always seen in stool, and these symptoms may last for 5-7 days (Leder *et al.,* 2011). If no medical attention is provided to the infected individual, it may lead to chronic giardiasis that presents with chronic diarrhea with malabsorption of fat (steatorrhoea) and malabsorption of vitamin A, protein and D-xylose. Malaise, nausea, anorexia, protuberance of abdomen, spindly extremities are also present, these, lasts for several weeks. Extra-intestinal are rare and sometimes urticarial and reactive arthritis are seen in rare case. If goes untreated, complication associated with giardiasis may be observed that includes, weight loss, disaccharides deficiency, malabsorption, and growth retardation (Dizdar *et al.,* 2007; Buret *et al.,* 2011).). Some patients may experience persistent symptoms (e.g., chronic diarrhea/steatorrhea, malabsorption) despite apparently effective antibiotic treatment, although these usually subside over weeks to months (Morken *et al.,* 2008).

Hanevik and colleagues (2009) found symptoms consistent with irritable bowel syndrome (IBS) and/or functional dyspepsia in 76 of 82 patients at least 6 months after eradication of *Giardia* infection characterized by bloating, diarrhea, and abdominal pain, which were exacerbated by specific foods or by physical or mental stress. Another study by Hanevik and

colleagues (2009) associated giardiasis with the presence of IBS and chronic fatigue even 6 years after infection. In children between 4-14 years *Giardia lamblia* caused a significant proportion of recurrent abdominal pain (Younas *et al.,* 2008). The prognosis for patients with giardiasis is generally excellent. Most patients are asymptomatic, and most infections are self-limiting. Giardiasis is not associated with mortality except in rare cases of extreme dehydration, primarily in infants or malnourished children (Robertson *et al.,* 2021). Several antibiotic, including metronidazole agents are available with good efficacy rates to shorten the disease course, although drug resistance has been observed in clinical experience. Untreated, giardiasis can last for weeks as the parasite can persist in the bowel and stool (Robertson *et al.,* 2021). A diagnosis of giardia infection can be delayed or missed for a variety of reasons. Asymptomatic infections are responsible for the continued transmission of the parasite as numerous cysts are produced. Individuals with diarrheic stools are passing primarily trophozoites which cannot survive in the environment (Ford, 2005).

2.3 Diagnosis of *Giardia lamblia*

This is performed through stool analysis and the first step is examination of the stool macroscopically and recording the findings, thereafter various diagnostic techniques: including microscopy, serological test, molecular techniques such as PCR can be employed.

2.3.1 Macroscopic Examination

A stool analysis is done if the medical history and symptoms show that one is suspected of having giardiasis. Stool sample procedurally is examined macroscopically for appearance, which can be mucoid or semi-mucoid, these characteristics guide the microscopist on the possible organism to expect in stool, these organisms may include parasites like cryptosporidium species, giardia species, and bacteria, like shigella species, salmonella species, fungal and viral organisms. Secondly, the color is reported, the color varies depending on what the individual has ingested, it can assume a black color, majorly after taking iron supplement or bismuth medication, green could be normal or too much intake of green leafy food, pale, white, or clay-colored meaning lack of bile, red may be due to blood or food like beets, cranberries, yellow caused by too much fat, or malabsoption disorder and finally, consistency can be formed, loose or watery. If the stool consistency can be determined it may give an indication of the types or stages of organisms present. Trophozoites (motile forms) of the intestinal protozoa are usually found in liquid specimens; both trophozoites and cysts might be found in soft specimens. Cyst forms are generally found

in formed specimens; however, there are always exceptions to these general statements (Garcia *et al.*, 2015).

2.3.2 Microscopy

The microscopic examination of the stool specimen, normally called the ova and cyst parasite examination, consists of three separate methods: the direct wet smear that primarily looks for trophozoite and their motility, the concentration methods, recover more organisms, in cyst form and the permanent stained smear (demonstrate detailed parasite morphology). *Giardia lamblia* exists primarily in two forms, cyst (dormant stage) and trophozoites (active stage) forms (Farghiri and Widmer, 2011). A number of morphological features of the trophozoite can be used in identifying the organism and they include; the shape which is pear shaped with broad round anterior end and a tapering posterior end, with a dorsal convex, and ventral concave surface (sucking disc also known as adhesive disc) which acts as an organ for attachment, behind the adhesive disc lies a pair of large curved and transverse median bodies (Guterrezi, 2017). Other morphological feature of importance includes, the presence of a cytoplasm that is uniform and finely granulated, with a typical 'fallling leaf type' motility (Adam, 2001).

A fully mature cyst appear as ovoid or ellipsoidal in shape measuring 8-12µm in length and 7-10µm in breadth, surrounded by a thick cyst wall, with a granulated cytoplasm and is separated from the cyst wall by clear space, a cyst has 4 nuclei, the remaining of flagella and the margins of sucking disc may be seen inside the cytoplasm (Gutierrezi, 2017).

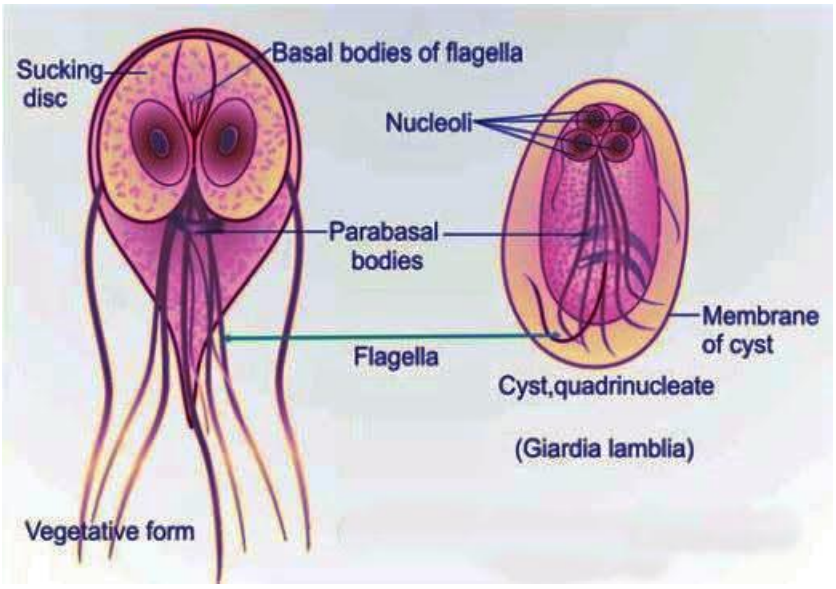

Figure 2.1: Trophozoite and cyst of *G. lamblia*

Staining techniques can be employed to aid in the detection of these stages of giardia, and their differentiation from other micro-organisms and fecal or environmental debris. The simplest stains include 1%Lugol iodine. Cysts can be concentrated using various methods which employ, for example, formalin-ether or formalin-ethyl acetate (Smith and Paget, 2007). Motile trophozoites can be detected by direct microscopic examination of fresh samples (smears prepared immediately with warm saline at 37 °C. Multiple, successive fecal samples should be taken and examined over a period of 1–2 weeks, because of the intermittent nature of *Giardia lamblia* cyst excretion (Garcia, 2009).

Each of these methods is designed for a particular purpose and forms an integral part of the total examination of stool for trophozoites, cysts, eggs, spores and oocyst of enteric organisms. Other substances of importance also found in fecal substrate include; Red blood cells (RBCs) which may indicate ulceration or other hemorrhagic problems, white blood cells (WBCs), polymorphonuclear leukocytes (PMNs), which may indicate inflammation, parasitic or bacterial infection, and presence of charcot-Leyden crystals which may be found when eosinophils are disintegrating. Finally, fungi, which mainly is Candida species, and other yeasts (Garcia *et al.,* 2015). These-afore mentioned reasons, make microscopy time-consuming and labor-intensive.

2.4 Molecular techniques

Convectional PCR enables the specific amplification of DNA regions from complex genomes. (Gasser, 2006). Numerous methods have been assessed (Adamska *et al.,* 2010), and include the use of the following techniques for the initial process of DNA extraction namely; sonication, freeze/thaw cycling, glass beads and/or phenol/chloroform extraction, followed by ethanol precipitation, and/or commercial extraction kits, such as QIAamp (Qiagen, USA). However, DNA isolation and purification methods require critical evaluation and expertise for each distinct application, and biological matrix being tested, in order to ensure that PCR-inhibition is minimised. DNA (genomic product) amplification normally is achieved by the following protocol: Several denaturation cycles at 95ºC each lasting about at least 30 sec, annealing at 65ºC for 30 sec and extension at 72ºC for 1 min and a final extension at 72ºC for 7 min. The final PCR products from the amplifications is usually visualized by electrophoresis on a 1.5% agarose gel containing ethidium bromide. Cross-contamination is common in a minority of cases because of the large amount of *G. lamblia* DNA present in most positive specimens (Schuurman *et al.,* 2007).

G. lamblia, which is found in humans and many other mammals, including pets and livestock, is now considered a multispecies complex that comprises at least eight distinct genetic groups, referred to as assemblages A to H (Ryan and Caccio, 2013), depending on gene glutamate dehydroganase (GDH) , *beta* giardin, elongation factor-alpha, triose phosephate isomerase(TPI) and small subunit rRNA (Jerlstrom-Hultquist *et al.*, 2010). Some of these assemblages can be classified even further into subtypes like for example; A-I, infects humans and animals (cats, dogs, livestock, deer, muskrats, beavers, voles, guinea), A-II affects humans (more common than A-I) A-III, exclusively for animals, A-IV, C and D for dogs, coyotes, E infects alpacas, cattle, goats, pigs, sheep, F for Cats (Xiao and Fayer, 2008). Glutamate dehydroganase (GDH) genes, triose phosphate isomerase (TPI) genes are commonly targeted, mainly because they are constitutive genes, with variation in sensitivity and specificity. Studies have shown GDH specific fragments amplication of upto 95% while TPI had 90% when microscopy was used as a gold standard, with (5′-TCAACGTCAACCGCTTCCT-3′) used as the primer (David *et al.*, 2011). Sensitivities of PCR based assay are generally high, this is as result of PCR ability to pick or detect minute genetic particle of an organism, however, there are possibilities of false result occasioned by large genomic quantities of the assayed organism. Though PCR has its limitations,PCR was used as a referrence procudure in this study, laboratory technique are generally compared with other test or tests that have an ability of producing reliable results, this is done primarily to check the functionality or accuracy of results of the gold standard and by extention the new test being introduced. (Garcia *et al.*, 2015).

2.5 Immunological techniques

Several immunologogical methods for Giardia species infection have been in use, these methods primarily rely on antigen/antibody reaction, this include the following examples, indirect immunofluorence (IIF) assay, IFF is a semi-quantitative, sensitive, and rapid test for the detection of immunoglobulin against *G. lamblia* in fecal specimen. This is where antibodies against the trophozoites are recovered and later the elutes mixed with specific giardia antigen with fluorescein isothiocyanate conjugates at different dilution ratios. The time required to perform the test is approximately 1.5 hours. Slides are read for the presence of antibody binding to a sample using a fluorescent microscope, and the intensity of signal fluorescence is graded. DFA tests have mostly used MAbs conjugated with fluorescent molecules for direct detection of *G. lamblia* in cellular smears. Direct fluorecent antibody(DFA) and indirect fluorecent antibody (IFA), the test detects the presence of a

particular antigen (typically a specific protein on the surface of a virus, bacterium or other microbes) (Kridin *et al.,* 2018).

Enzyme-linked immunosobent assay (ELISA), may be done according to (Miottiet *et al.,* 1985), where it can be direct, indirect or sandwich ELISA, for detection of specific giardia antigen or in the detection of anti-giardia antibodies produced against *G.lamblia*, ELISA combine the specificity of antibodies with the sensitivity of simple enzymes assays, by using antibodies or antigens coupled to an easily-assayed enzymes. Enzyme-linked immunosorbent assays (ELISAs) are plate-based assays for detecting and quantifying a specific protein in a complex mixture. The detection and quantification of target-specific protein in a sandwich ELISA is accomplished by using highly specific antibodies that immobilizes the target protein (antigen) to the plate and indirectly detects the presence of the target protein. This type of capture assay is called a sandwich assay because the analyte being measured is bound between two primary antibodies, each detecting a different epitope of the antigen - the capture antibody and the detection antibody. This procedure involves several washing of the plate and incubation of the plate, Which is time consuming (Rishniw *et al.,* 2010). Another available test used in diagnosis of Giardia is enzyme immunoassays (EIA), majorly these methods uses enzymes labelled antibodies and antigens to detect the small biological molecules required, the techniques makes use of the basic immunology concept that an antigen binds a specific antibody (Halson and Cartwight, 2001). These methods can be time consuming i.e some requires overnight incubation or atleast 30 minutes incubation at variuos levels of the test according to the manafacture, with several washes of the plates/ tubes, and furthermore all require training and experience to achieve reliable results (Wilson and Hankenson,2011).

Immuno-chromatographic methods can be advantageous over light microscopy, which is the gold standard, for the detection of Giardia cysts or trophozoites in biological samples. One such approach is the detection of Giardia antigens in faecal samples (i.e., giardia RDT) ,is one of the approach by immuno-chromography (IC) tests (Costache *et al.,* 2009). This test is ready to use and is based on the homogeneous membrane system technology with colloidal gold particles. This device allows detection of *Giardia lamblia* in stool specimen. The specificities come from monoclonal antibodies directed against *Giardia lamblia*. When three drops of a mixture of buffer and fecal matter are placed into the sample area on a test device, conjugates dried in the application membranes located on one sides of the cassette are solubilized and migrate along with the sample i.e. the cassette has purified antibodies against

giardia on test band and purified giardia antigen on the control band. Various test kits have been in use for screening small population for giardiasis, these kits may have the same test principle as that of SPANBIO^{TM} RDT, but the test protocol could be different i.e. when mixing the stool sample with the buffer, and the allowed time given to read the results, some can take up-to 30 minutes for a result to be recorded, and furthermore, majority are RDT strips, which allows room for self-contamination, but for SPANBIO^{TM} RDT, the test protocol is simple as described in the methodology section, if the sample contains *Giardia lamblia*, a complex will be formed with the anti-Giardia conjugate and it will be caught by the Giardia monoclonal antibody coated. Results appear in 10 minutes in the form of a red line that develops on the strip, and produced as a cassette (SPANBIO, 2015). Advantages that giardia antigen detection methods offer over microscopy are that they have the capacity to detect (prepatent) infections prior to the excretion of cysts in host faeces, and can be employed for the cost-effective and rapid screening of large numbers of faecal samples (Garcia *et al.*, 2003; Geurden *et al.*, 2008b; Johnston *et al.*, 2003). However, factors such as intermittent shedding of cysts and Giardia antigens can reduce sensitivity(Reynoso-Robles *et al.*, 2015). Decreased sensitivity might also relate to low numbers of cysts in faeces (Strand *et al.*, 2008).

2.6 Specificity

Specificity of a clinical test (diagnostic test) refers to the ability of the test to, correctly identify those patients without the disease (Lalkhen *et al.*, 2008). Various test have different specificity. Estimating the specificity of a test is done by identifying non infected individuals with (microscopy) gold standard and dermining false positive of the new diagnostic test. Specificity of a test can be affected by, characteristics of the non-infected persons, which may include, the presence of circulating antibodies, which can cause cross-reactivity. For example if malaria is endemic, polyclonal hyper gammaglobulinemia may increase the proportion of false positives, therefore, study design and the enviroment where the study population leave can have a major effect. (Mostafavi, 2017). Negative Predictive value (NPV), is the probability that an individual testing negative is truly non-infected, and can be affected by sensitivity, prevalence of the disease, i.e the test will pick up more true negative in an area with low prevalence, and in high prevalence, the test will pick up more false negatives (Mostafavi, 2017). Microscopy, which is the gold standard largely depends on the skills of the microscopist, in identification of normal specimen debris and, other fecal material. According to a study done by (Elnahas *et al.*, 2012), ordinary microscope had a specificity of

100% and NPV of only 72.7%. Validation test of SPANBIO^{TM} RDT achieved specificities of between 79–100% and negative predictive value of at least 99% depending on study (Johnston *et al.,* 2003). Validation in a laboratory is done by testing few samples, normally not more than ten samples, with a test that was previsouly used. Specificity of SPANBIO^{TM} detection assays can be lower than microscopic approaches (Johnston *et al.,* 2003). Other kits that have shown specificities of between 92.1-100% are; Rida quick, in this procedure, a small portion of stool is placed in an ampuole containing PBS buffer, and mixed well by a vortex, thereafter a strip of the Ridaquick is placed in the mixture, the results are ready after 15 min, Duo strip, the procedure is also the same as for Ridaquick but in this test the PBS buffer is added in a test tube that has about 2gms of feacal sample and the RDT strip is held vertically with the end of the pad touching the mixture, the results are ready after 15min. RIDASCREEN, has several steps including PBS buffer prepation, washing sereval times the mixture and then incubation follows, this may be time consuming and finally, Quick check, this RDT has two test for cryptosporidium species and giardia species but manafuctured as a strip which is insterted into a tube containing a mixture of stool and PBS buffer, the results are ready after 30 minutes. These mentioned kits or technique use similar principle to that of SPANBIO^{TM} RDT (Dyad *et al.,*2016; Abdul-Mumin *et al.,* 2019). Therefore, the proposed study determined specificity of SPANBIO^{TM} against microscopy and PCR in detecting *G. lamblia* from stool specimen that contains various organic and inorganic matters.

2.7 Sensitivity

The sensitivity of a clinical test refers to the ability of the test to correctly identify those patients with the disease (Lalkhen *et al.,*2008). This involves identifying infected individuals with microscopy which is a gold standard . Sensitivity can be influenced by antigenic characteristics of the pathogen in the area e.g., if the test was not prepared with antigens reflecting the population of pathogens in the area, it will not pick up infected persons in the area. Positive Predictive value is the probability that an individual testing positive is truly infected, and can be influenced by specificity i.e the more the test is specific, the more it will be negative for non affected persons, thus, when the test is positive, it is probably truly positive , secondly, the prevalence i.e when the prevalence is low, the test will pick up more false positives, and when the prevalence is high, the test will pick up more true positives. Study design and the enviroment where the study population leave can have a major effect. (Mostafavi,2017).The sensitivity of microscopy(gold standard) also largely depends on the skills of the microscopist (Rosenblatt *et al* 1993), ordinary microscopy had a sensitivity of

76.9%, with PPV of 100%. Validation test of SPANBIO™ RDT achieved sensitivities of up to 100%, with positive predictive values of between 90.5-100%, which also depended on the study (Johnston *et al.,* 2003). Therefore, the sensitivity of SPANBIO™ detection assays can be higher than microscopic approaches (Johnston *et al.,* 2003). Other kits like Rida quick, Duo strip, RIDASCREEN, Quick check, have shown sensitivities of between 58-100% (Bossche *et al.*,2016; Bitilinyu-Bangoh *et al.*,2019). RDT have shown prozone effect as a result of over-production of immunoglobulin after infection, and this was one of the limitations (weitzel *et al.,* 2006). These test all are immuno-chromatographic, that highly rely on antigen and antibody reaction. Therefore, the proposed study determined sensitivity of SPANBIO™ against microscopy and PCR in detecting *G.lamblia* from stool specimen that contains various organic and inorganic matters.

2.8 Organic and inorganic composition of fecal substances and effect on test performance

Variation in diagnostic performance of different assays can be due to cross-reactivity (affecting specificity) these may be caused by organic compounds like, fats, undigested starch or inorganic compounds for example protein,shaded epithelial and pus cells, these compunds may appear naturally or under diseased condition (Reynoso-Robles *et al.,* 2015). Other factors are use of formalin as a fixative (reducing sensitivity) and or altering the pH of stool (Reynoso-Robles *et al.,* 2015). Decreased sensitivity might also relate to mixed infection with other intestinal parasites. Alcohol may interfere with test result of most RDT. Therefore, it is not recommended to use RDT for diagnosing various conditions after drinking alcohol or after using alcohol based medicine (Reynoso-Robles *et al.,* 2015). Mixed infection observed in fecal content may also have some effect on the test performence ,therefore, this study determined clinical factors that interfered with SPANBIO™ RDT test performance in diarrheal and dysentric patients.

2.9 Treatment and prevention of *G. lamblia*

Giardiasis can be treated and managed by single dose of metrinidazole of diffent strength depending on body weight or a combination of drugs, such as quinacrine among both immunocompromised and healthy patients with giardiasis (Nash *et al.,* 2001). Prevention of Giardia infection can often be done by practicing simple hygiene, observing drinking water precautions and treatment of giardia infection (CDC, 2015).

CHAPTER THREE
MATERIAL AND METHODS

3.1 Study area

The study was conducted at Busia County Referral Hospital. Busia County covers an area of 1,695 Km^2 (628.7 sq. miles), and is located in the Western part of Kenya, with its boundaries marking the Kenya-Uganda boarders to the west (Fig 1). The County borders three other counties which include: Bungoma to the north, Kakamega to the east and Siaya to the south west. Part of Lake Victoria is in the County on the South East. It lies between latitude 0º and 0º 45 north and longitude 34º 25 east. Busia County has seven sub- county hospitals namely Matayos sub- county hospital, Butula sub-county hospital, Sio Port sub-county hospital, Port Victoria sub-county hospital, Nambale sub-county hospital, Teso south sub -county hospital, and Teso North sub -county hospital. Busia County has a total population of 743,946 (KNBS, 2009).

The county's population was made up of Females at 53.1%, males 47.9% respectively and 17.7% children below the age of five. By the year 2017, the population was estimated to have grown to a total of 953,337(456,356 males, 496,981 females, and 168, 862 of children below the age of five) (KNBS, 2009). Busia County has equatorial climate and there are two rain seasons in the county long rains and short rains. There is one major river: River Sio. The main economic activity was agriculture and livestock farming. Main crops grown are: maize, sugarcane and horticultural crops. The hospitals serve all people from different economic status and a majority of the population in the county live below poverty line (KNBS, 2009).

BUSIA COUNTY
DISTRIBUTION OF HEALTH FACILITIES

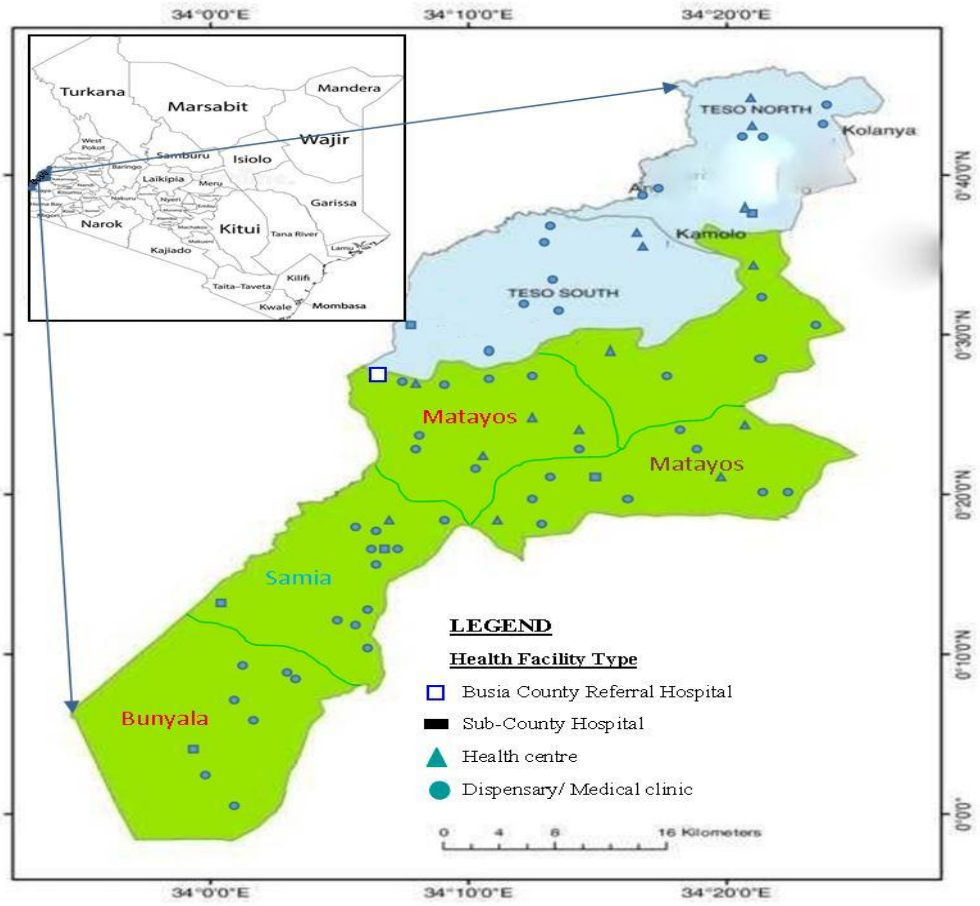

Figure 3.1: study area

3.2 Sample population

The study population were all individuals sent to the laboratory at the Busia County Referral Hospital with gastrointestinal manifestations and sample population consisted of individuals with cardinal sign and symptoms of giardiasis which include bloating, burping, diarrhea that was greasy or blood stained, and abdominal pain.

3.3 Study design

The study was a cross-sectional health facility-based study.

3.4 Sampling design

Non-probability convenient sampling design was employed and 139 individual with cardinal signs and symptoms of giardiasis, were recruited. The sign and symptom were; Loss of

appetite, diarrhea, loose or watery stools, stomach cramps, stomach upset, bloating and excessive gas.

The following formulae, according to Cochran *et al.,* (1963) was used.

$$n_o = \frac{z^2 pq}{e^2}$$

Where n_o = Sample size (139)

Z = desired confidence level $(1.96)^2$

p = Estimated proportion of an attribute present in a population (0.10). Previous studies indicated a prevalence of symptomatic giardiasis in former western province at 10.0 % (Brett *et al.,* 2012).

q = 1 – p (0.90)

e = Desired level of precision $(0.05)^2$

$$\text{Sample Size} = \frac{(1.96)^2(0.10)\ (0.90)}{(0.05)^2}$$

= 139

The sample was adjusted by 10% to carter for non-response, the final sample size was 147.

3.5 Inclusion and exclusion criteria

3.5.1 Inclusion criteria

Individuals presenting any of the following cardinal signs and symptoms of giardiasis; loss of appetite, diarrhea, loose or watery stools, stomach cramps, stomach upset, bloating and excessive gas, and able to produce at least 2gms of stool, all age group of any gender and consented to participate in the study.

3.5.2 Exclusion criteria

Individuals with fistula. This can result to mixing of fecal content and urine, affecting sample quality by introducing chemicals that can affect the pH of the test device. Individuals who had a confirmed *G. lamblia* results and are on treatment or are from treatment.

3.6 Methods of data collection

At time of specimen collection, patients and/or their parents completed written questionnaires that were critical in finding the sample population. Standardized questionnaires covering Demographic data, previous medications, cardinal signs and symptoms of giardiasis were used. On the laboratory results forms the stool results entries were made after the stool had

been examined by a qualified and certified stool slide reader. Thereafter there was dual data entry of all indicators first to an excel spread sheet. Samples were collected from 25th September 2017 to 30th June 2018.

3.7 Laboratory procedures

3.7.1 Collection of stool samples for analysis

Fecal matter was obtained after carefully instructing patients to defecate and put a small amount of feces in a poly-pot using the provided spatula. Thereafter, stool analysis by Macroscopy and microscopy followed the same day, which was, direct method.

3.7.2 Macroscopic and microscopic analysis of stool

The poly-pot was carefully opened and the following observations were indicated; Consistency, whether the sample was mucoid, semi-mucoid, color of the sample. The direct wet smear was prepared by mixing a small amount of stool (about 2 mg) with a drop of 0.85% NaCl; this mixture provided a uniform suspension under a 22-by 22-mm coverslip. A 2-mg sample of stool forms a low cone on the end of a wooden applicator stick. If a fresh stool specimen was received and if blood and mucus are present, the specimen was examined as a direct mount making sure to sample the bloody areas. The entire 22- by 22-mm coverslip was systematically examined with the low power objective (10x) and low light intensity; any suspicious objects were then examined with the high dry objective (40x). When the cyst were seen, one drop of Lugol's iodine was added on the slide, then the slide examined systematically for cyst, larvae and ova using the x10 objective. Cyst and ova were examined in more detail using x40 objective and observations recorded (AMREF SOP, 2009).

Thereafter a small proportion of approximately 1 gm stool was placed in each of the three eppendorf tubes, one eppendorf tube was empty, for the SPANBIO™ test, while the remaining two had 0.3 1ml each of potassium dichromate (preservative) and stored in -20 freezer for DNA extraction (Kuk *et al.*, 2012). The remaining feces was discarded in yellow bin (labeled infectious) for incineration (MOH Laboratory Biosafety and Biosecurity Training participants' manual 2016).

3.7.3 Spanbio™ RDT

The kit was brought to room temperature, to improve the performance of the device, this is important as component impregnated in the kit works well at a range of temperatures. The patient unique number was labeled on the ampoule containing the PBS buffer, the upper part of the ampoule was gently opened and a small amount, approximately 1gm of feces was placed into the ampoule and tightened, the mixture was mixed by shaking vigorously to

achieve homogenized solution thereafter a drop of the solution was placed on the test area and results read after 10 minutes. Results were interpreted as follow, 1 line (upper) = negative, 2 lines = positive, 1 line (lower) = invalid, 0 line= invalid. The absence of migration control line (at any side), which is the upper line, makes the result invalid. In this case, the sample must be retested (SPANBIO Co., 2015). This kit only targets giardin antigen in a fecal sample, and since the kit has giardin antibodies on the test band and only bind to specific antigen in feces, chances of cross-reactivity are significantly reduced. This current study ensured that the RDT was kept in optimum conditions and exposed when the test was about to be performed.

3.7.4 Total DNA extraction

A total of 78 samples identified as positive and 69 samples identified as negative for *Giardia* by microscopy were processed for extraction of genomic DNA using QiAmp® DNA stool Mini kit (Qiagen, Crawley, West Sussex, UK) and following the manufacturers protocol. Briefly, about 200µL sample suspension was added to 20µL protease in a micro-centrifuge tube. About 200µL of buffer AL was added to the sample, mixed and incubated at 56°C for 10 minutes. About 200µL of ethanol (96-100%) was added to the sample, mixed and centrifuged at 8000 rmp for 1minute. The mixture was then transferred to spin column, centrifuged at 8000 rpm for 1 minute and the filtrate discarded. About 500 µL of buffer AW1 was added to the spin column placed in a fresh 2ml collection tube, centrifuged at 8000rpm for 1 minute and the filtrate discarded. The spin column was placed in a fresh collection tube and centrifuged at 18000 rpm for 1 minute and the filtrate discarded. Finally, DNA elution was performed by addition of 200 µl buffer AE to spin column in a fresh 2ml collection tube, followed by incubation at room temperature for 1 min, and then centrifugation at 8000 rpm for 1 minute. The resulting DNA was aliquoted and stored at −20 °C until the laboratory assays were performed (Kuk *et al.,* 2012).

3.7.5 PCR product purification

PCR amplicon was cleaned using DNA Purification Kit according to the manufacturer's protocol (QIAquick™ Inc., Valencia, USA). About 75 µl of Buffer PBI was added to PCR product and mixed. The mixture was transferred to spin column placed in a 2 mL collection tube, centrifuged for 1 minute and the filtrate discarded. About 0.75 ml Buffer PE was added to the spin column in the 2 mL collection tube, centrifuged at 1800rpm for 1 minute and the filtrate discarded. DNA elution was performed by addition of 30 µL Buffer EB (10 mMTris-

Cl, pH 8.5) to the spin column in a fresh 1.5 ml micro-centrifuge tube, followed by centrifugation for 1 minute.

3.7.6 PCR

Prior to the PCR amplifications, the DNA concentration of each sample was measured using a Nanodrop (ACTGene ASP-3700, USA) instrument. After this measurement, PCR was performed with primer (5′-TCAACGTCAACCGCTTCCT-3′) 432-bp region which targeted the GDH gene (Cacciò *et al.* 2002). The PCR amplifications were each performed in a 25 μL volume that contained 12.5 μL 2x PCR Master Mix (Vivantis, Malaysia), 2 μL of each primer (at 20 pmol) and 5 μL of genomic template DNA. These reactions were performed in a PCR Labcycler (SENSQUEST, Germany). The following PCR cycle was as follow: an initial denaturation at 95ºC for 5 min, 35 subsequent cycles of denaturation at 94ºC for 30 sec, annealing at 65ºC for 30 sec and extension at 72ºC for 1 min and a final extension at 72ºC for 7 min. Negative controls for the amplification reaction mixtures that contained only the PCR reaction reagents and sterile distilled water was prepared. PCR products from all of the amplifications was visualized by electrophoresis on a 1.5% agarose gel containing ethidium bromide and the gel image was recorded under trans-illuminator UV light. (Kuk *et al.* 2012). PCR, being a molecular test, was used as a referrence procudure in this study, mainly to guide the researcher in checking the performance of the gold standard.

3.8 Data analysis

Statistical analysis was performed using SPSS, version 24.0 (IBM, Chicago, USA). Data was summarized by proportions, mean and presented as tables. Analyses included sensitivity, specificity, and positive and negative predictive values using the following formulas below.

Formula to calculate specificity

Specificity is the ability of a test to detect people who do not have disease, as determined by a gold standard.

$$\text{Specificity} = \frac{TN}{TN+FP} \times 100$$

Formula to calculate sensitivity

Sensitivity is the ability of a test to detect people who truly have the have disease, as determined by a gold standard.

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100$$

Formula to calculate PPV

$$PPV = \frac{TP}{TP+FP} X100$$

Formula to calculate NPV

$$NPV = \frac{TN}{TN+FN} X100$$

Factors influencing SPANBIO™ RDT positivity were analyzed using Pearson chi-square. Agreement between specificity and sensitivity of SPANBIO™ RDT with microscopy and PCR was determined using Cohen kappa analysis. Proportions of *Giardia lamblia* distribution amongst groups was examined using Pearson chi-square. Regression was done to establish any association between various factors and performance of SPANBIO™ RDT. All tests were two-tailed and statistical significance was determined using *P*-value of less than 0.05.

3.9 Ethical consideration

Approval to carry out this study was provided by School of Graduate Studies (SGS) of Maseno University. Ethical approval was obtained from Maseno University Ethical Review Committee. Thereafter, Busia county referral hospital administration approved the request to commence the study at the facility. Written informed consent was obtained from each participant before enrolment. Confidentiality was maintained during the study by assigning the participants with unique code numbers. Study participants who were found to be infected with *Giardia* and other intestinal protozoa and helminthes were treated according to national guidelines for intestinal protozoa and helminthic treatment. This included participants for *Giardia lamblia* and protozoa, and participants for helminthes, treatment was administered by a qualified and registered clinician according to the ministry of health guidelines. This included administration of tinidazole for giardiasis and other intestinal protozoa, and anti-heliminths for helminths. All study documents was archived in secure cabinets. Access to the data was limited only to the investigators. The participants benefited with free health education on intestinal infections, risk factors and prevention, hygiene and hand washing.

CHAPTER FOUR

RESULTS

4.1 Demographic, laboratory and clinical characteristics of the study participants

In this study 147 individuals were recruited. Table 4.1. Individual of 0 years of age and older were enrolled in the study, and n=78(mean age 23.9, range: 0.01-73.1 years) tested positive. Of the 147 individual n=69 (mean age 25.3, range: 0.09-74.8 years) tested negative, p = 0.244. Female and male were statistically different: 29[37.2%] females and 49 [2.8%] males were among the positive, and 41 (59.4%) females and 28(40.6 %) males were among individual who tested negative, p = 0.008.

Additional information of importance, and which add more information to the body of knowledge used by clinicians and nurses on who to be done what test depending on the signs and symptoms presented by a patient, and to the laboratory officer, in selecting appropriate sample revealed the following under clinical presentation associated with *Giardia lamblia* infection, those with poor appetite were 95(30[31.6%] among the positives, and 65[68.4%] among the negatives, p = 0.152). Those presenting with vomiting were 39(16[41.0%] among the positives, and 23[59.0%], p =0.560). Participants experiencing flatulence were 35(12[34.3] among the positives, and 23 [65.7%] among the negative, p = 0.843).Those with headache were 47(24[51.1%] among the positives, and 23[48.9] among the negatives, p = 0.016). Among the recruited and experiencing blotting were 63(36[57.1%] among the positives, 27[42.9%] among the negatives, p < 0.0001. Those that had stomach discomfort were 64(21[32.8%] among the positives, 43[67.2%] among the negatives, p =0.494).Those experiencing abdominal pain 92 (31[33.7%] among the positives, and 61[66.3%] among the negatives, p =0.480). Those presenting with diarrhea were 32(41.0%) and without diarrhea were 46(59.0%) among the negatives, while among the negatives 9(13.0%) had diarrhea and 60(87.0%) had no history of diarrhea, p<0.0001. Participants presenting stool with foul smell were 48(31[64.6%] among the negatives, 17[35.4%] among the positives, p <0.0001). Those producing fatty stool were 11(8 [72.7%] among the positives, and 3[27.3%], among the negatives, p = 0.018).

While those producing mucus in stool were 34(25[73.5%] and 9[26.5%], among the individual who tested negative and positive respectively, (p <0.0001). Those producing whitish stool among individuals were 12 (10[83.3%] among the participants who tested positive, and 2[16.7%] participants who were negative, p = 0.001). Those that had presence of pus cells in their stool were 31(22[71.0%] among the positive, and 9[29.0%] among the

negative, p <0. 0001). Those producing formed stool were 44(1[2.3%] among the positive, and 43[97.7%] among the negative, p <0. 0001). Participants producing semi-formed stool among the group that tested positive were 53(26[49.1%], and 27[50.9%] among the negative, p = 0.020). While those presenting with loose stool were 49(25[51.0%] among the positive, and 24[49.0%] among the negative p = 0.011). Laboratory results of fecal examination showed that there were a total of three samples that had mixed infections, that is, two stool samples had *Entamoeba hystolitica* cysts and, one had *Trichuria Trichuris* cyst.

Table 4.1: Demographic, clinical characteristics and laboratory findings of the study participants

Variable	**Positive cases, n=78**	**Negative cases, n=69**	X^2	p
Age, (range) years	23.9 (0.01-73.1)	25.3 (0.09-74.8)		0.244
Gender, n (%)				
Female	29 (37.2)	41 (59.4)[a]		0.008
Male	49 (62.8)	28 (40.6)		
Poor feeding	30 (31.6)	65 (68.4)[b]	**2.333**[a]	0.152
Vomiting	16 (41.0)	23 (59.0)[b]	**0.569**[a]	0.560
Flatulence	12 (34.3)	23 (65.7)[b]	**0.062**[a]	0.843
Headache	24 (51.1)	23 (48.9)[b]	**6.751**[a]	0.016
Bloating	36 (57.1)	27 (42.9)[b]	**21.267**[a]	**<0. 0001**
Stomach upset	21 (32.8)	43 (67.2)[b]	**0.517**[a]	0.494
Abdominal pain	31 (33.7)	61 (66.3)[b]	**0.593**[a]	0.480
Diarrhea				
Yes	32 (41.0)	9 (13.0)[a]	**21.900**[a]	**<0.0001**
No	46 (59.0)	60 (87.0)		
Foul smelling stool	31 (64.6)	17 (35.4)[b]	**25.161**[a]	**0.0001**
Fatty stool	8 (72.7)	3 (27.3)[b]	**6.936**[a]	0.018
Mucus stool	25 (73.5)	9 (26.5)[b]	**26.942**[a]	**<0.0001**
Stool color	10 (83.3)	2 (16.7)[b]	**12.669**[a]	**0.001**
Pus cells	22 (71.0)	9 (29.0)[b]	**20.770**[a]	**<0. 0001**
Consistency				
Formed	1 (2.3)	43 (97.7)[b]	**31.083**[a]	**<0. 0001**
Semi-formed	26 (49.1)	27 (50.9)[b]	**6.078**[a]	0.020
Loose	25 (51.0)	24 (49.0)[b]	**7.141**[a]	0.011

Data presented are number and proportions (%) of subjects, age is shown as mean (range) years, [a] denotes

Figure within, and [b] denotes figures across

4.2 Specificity and sensitivity

Of the 78(53.1%) participant microscopically confirmed positive to *Giardia lamblia*, SPANBIO^{TM} RDT revealed 52, with 1(1/69) that tested positive by SPANBIO but negative microscopically, therefore a total 53(36.1%) from 147 participant tested positive on SPANBIO. Sensitivity and specificity of SPANBIO^{TM} was 66.7% (95% CI :55.1-76.9%) and 98.6% (95% CI:92.3-100%), respectively, while positive and negative predictive value was 98.1% (88.1-99.7%) and 72.3% (65.6-78.1%) respectively, with accuracy levels of 81.6% (74.4-87.5%), Cohen kappa 0.6388(0.0592,0523-0.755), and Mc Neymar test results of 17%(10.7-23.4; p<0.0001). There were 26(26/78) who were positive by microscopy but tested negative by SPANBIO, while 68 samples showed an agreement negative test results on both microscopy and SPANBIO, overall total of the negative samples tested by SPANBIO being 94(63.9%).

Glutamate dehydrogenase (GDH)-PCR was able to detect 61 samples from the 78 samples, confirmed microscopically as having *Giardia lamblia* , while 7 showed concordance result, where PCR was positive while microscopy was negative ,the total number of samples tested by PCR as being positive was 68(46.3%). Sensitivity and specificity of GDH-PCR was 78.2% (95% CI :67.4-86.8%) and 89.7% (95% CI:80.2-95.8%), respectively, while positive and negative predictive value was 89.7% (81.0-94.7%) and 78.5% (70.8-84.8%) respectively, with accuracy levels of 83.7% (76.7-89.3%), Cohen kappa 0.6750(0.06014,0557-0.7929), and Mc Nemar test results of 6.8%(0.4-13.2; p=0.169).There were 17(17/78) who were positive by microscopy but tested negative by GDH-PCR, while 62 samples showed an agreement test results on both microscopy and GDH-PCR, overall total of the negative samples tested by GDH-PCR being 79(53.7%).

Table 4.2 Specificity, sensitivity, predictive values and accuracy of SPANBIO™ rapid diagnostic test in the diagnosis of Giardia lamblia infections

				Sensitivity (95% CI)	Specificity (95%CI)	Predictive value		Accuracy		Methods agreement (Kappa, 95%CI)	Mc Nemar's (95% CI; Exact Probability)
						Positive Test (95%CI)	Negative Test (95%CI)	(95% CI)			
	Stool microscopy										
	Positive	Negative									
SPANBIO				66.7% (55.1-76.9%)	98.6% (92.3-100%)	98.1% (88.1-99.7%)	72.3% (65.6-78.1%)	81.6% (74.4-87.5%)		0.6388 (0.0592, 0.523-0.755)	17.0%
Positive	52	1	53 (36.1%)								(10.7-23.4;P<0.0001)
Negative	26	68	94 (63.9%)								
	GDH-PCR										
SPANBIO				78.2% (67.4-86.8%)	89.7% (80.2-95.8%)	89.7% (81.0-94.7%)	78.5% (70.4-84.8%)	83.7 (76.7-89.3%)		0.6750 (0.06014, 0.557-0.7929)	6.8%
Positive	61	7	68 (46.3%)								0.4-13.2;P=0.169
Negative	17	62	79 (53.7%)								
	78 (53.1%)	69(46.9%)	147 (100%)								

Data presented as proportions (%) with 95% confidence intervals (CI) unless otherwise indicated. SPANBIO™ RDT, PCR, polymerase chain reaction. PPV, positive predictive values. NPV, negative predictive values, accuracy. Cohen kappa agreement SE, McNemar's test

4.3 Factors influencing SPANBIO™ RDT performance

While evaluating the performance of SPANBIO™ rapid diagnostic test in detecting *Giardia lamblia* infections, the following variables were analyzed to determine their frequencies within the RDT positive and negative results, and the *p* value determined by fisher's exact test. Table 4.3. These factors affects the body's performance and eventually, affecting the physical, biological and chemical composition of stool, and these can be appreciated when feacal sample is observed macroscopically and microscopically. The following variables (factors) were analyzed. Participant who were feeding poorly were 95/147(30 were RDT positive and 65 were RDT negative) and 23[44.2%] with RDT positive results, and 29[55.8%] with RDT negative results, were not feeding poorly, (p=0.152). Vomiting among participants was, 16(RDT positive) and 23(RDT negative) while those with no symptoms of vomiting were 108(37[34.3%] RDT positive, and 71 [65.7%] RDT negative, p = 0.560). Those experiencing flatulence were 12 and 23, RDT positive and negative respectively, 41[36.6%] RDT positive, and 71[63.4%] RDT negative, p = 0.843) had no symptoms of flatulence. Those with headache were 24 RDT positive individuals and 23 RDT negative individuals, 29[29.0%] RDT positive, and 71[71.0%], p=0.016, were among those not with headache. Those presenting with blotting were 36 (RDT positive) and 27(RDT negative), while those with no bloating symptoms were 17[20.2%] RDT positive, and 67[79.8%] RDT negative, p <0. 0001). Individuals with stomach upset were 21(RDT positive) and 43(RDT negative) while, individuals with no stomach upset were 83(32[38.6%] RDT positive and 51[61.4%] RDT negative, p = 0.494).

Those experiencing abdominal pain were 31(RDT positive) and 61(RDT negative) and those with no abdominal pain were 55(22[40.0%] RDT positive and 33[60.0%] RDT negative, p = 0.480). Those experiencing diarrhea were 27(RDT positive) and 14(RDT negative) while those with no diarrhea were 106(26[24.5%] RDT positive, and 80[75.5%] RDT negative, p <0.0001).Those presenting stool with foul smell were 31(RDT positive) and 17 (RDT negative) and individual with no foul smelling stool were 99(22[22.2%] RDT positive, and 77[78.2%] RDT negative, p <0.0001). Those with fat in stool were 8(RDT positive) and 3 (RDT negative) while those with no fatty stool were 136 (45[33.1] RDT positive, and 91[66.9%] RDT negative, p = 0.018).Among the 113 participants who presented stool with mucus were 25(RDT positive) and 9(RDT negative), while those with no mucus were 28(24.8%) were RDT positive, while 85(75.2%) RDT negative, (p <0.0001). Participant producing whitish color stool (signifying

presence a possibility of fatty stool) were 10(RDT positive) and 2(RDT negative) with 135(43[31.9%] RDT positive, while 92[68.1%] RDT negative, p =0.001) had no white colored stool. Total participants with pus cell in stool were 22(RDT positive) and 9(RDT negative), while those with no pus cell were 116, 31[26.7%] RDT positive, and 85[73.3%] RDT negative respectively, p <0. 0001).

The study also sought to determine trend of RDT results among the three types of stool consistency namely; formed, semi-formed and loose and the following results were obtained in formed stool, 1(RDT positive) and 43(RDT negative) and 103(52[50.5%] RDT positive, while 51[49.5%] RDT negative, p <0. 0001), among the non-formed stool. In semi-formed, there were 26(RDT positive) and 27(RDT negative), while those with no semi-formed stool were, 27[28.7%] RDT positive, and 67[71.3%] RDT negative, p = 0.020), among loose stool 25(RDT positive) and 24(RDT negative) while those with no loose stool were, 98(28[28.6%] RDT positive, and 70[71.4%] RDT negative, p =0.011).

Table 4.3: Clinical and laboratory characteristics associated with SPANBIO™ RDT test results

Variable		RDT positive	RDT negative	X^2	*p value*
Poor feeding-	Yes	30	65		
	No	23 (44.2)	29 (55.8)	**2.333**[a]	0.152
Vomiting-	Yes	16	23		
	No	37 (34.3)	71 (65.7)	**569**[a]	0.560
Flatulence-	Yes	12	23		
	No	41 (36.6)	71 (63.4)	**0.062**[a]	0.843
Headache-	Yes	24	23		
	No	29 (29.0)	71 (71.0)	**6.751**[a]	0.016
Blotting-	Yes	36	27		
	No	17 (20.2)	67 (79.8)	**21.267**[a]	**<0. 0001**
Stomach upset-	Yes	21	43		
	No	32 (38.6)	51 (61.4)	**0.517**[a]	0.494
Abdominal pain-	Yes	31	61		
	No	22 (40.0)	33 (60.0)	**0.593**[a]	0.480
Diarrhea-	Yes	27	14		
	No	26 (24.5)	80 (75.5)	**21.267**[a]	**<0.0001**
Foul smelling stool-	Yes	31	17		
	No	22 (22.2)	77 (78.8)	**25.161**[a]	**0.0001**
Fatty stool-	Yes	8	3		
	No	45 (33.1)	91 (66.9)	**6.936**[a]	0.018
Mucus stool-	Yes	25	9		
	No	28 (24.8)	85 (75.2)	**26.942**[a]	**<0.0001**
Stool color-	Yes	10	2		
	No	43 (31.9)	92 (68.1)	**12.669**[a]	**0.001**
Pus cells-	Yes	22	9		
	No	31 (26.7)	85 (73.3)	**20.770**[a]	**<0. 0001**
Consistency					
Formed-	Yes	1	43		
	No	52 (50.5)	51 (49.5)	**31.083**[a]	**<0. 0001**
Semi-formed-	Yes	26	27		
	No	27(28.7)	67 (71.3)	**6.078**[a]	0.020
Loose-	Yes	25	24		
	No	28 (28.6)	70 (71.4)	**7.141**[a]	0.011

Data presented are number and proportions (%) of subjects

Further analysis on stool characteristics. Table 4.4 Showed that participants producing formed stool and tested negative and positive were 103 (70.1%) and 44(29.90%), respectively with OR(odds ratio) of 1.061 at 95% CI (0.856-1.069%)], $p = 0.095$, while those presenting semi-formed

stool and tested negative were 94 (63.90%), with the positive ones being 53(36.1%) [OR 0.162 (95% CI: 0.508-1.431%)], p = 0.871, participant with loose stool and tested negative were 98 (66.70%), with those testing positive being 49(33.3%) [OR 0.814 (95% CI: 0.659-1.275)], p = 0.417. Fecal pus cell among participants' stool who tested negative was 116 (78.1%), and those tested positive were 31(21.9%)[OR 2.318 (95% CI: 0.035-0.439)], p = 0.022, and those presenting stool with mucus and tested negative and positive were 113 (76.90%) and 34(23.10%), respectively with OR(odds ratio) of 2.982 at 95% CI (0.089-0.440), p = 0.003, participants presenting stool that had brown color and tested negative were 135 (91.80%) and those that tested positive were 12(8.2%) [OR 1.077 (95% CI 0.475-1.026)] p = 0.078, while those presenting stool with fat and tested negative were 136 (92.5%), with 11(7.50%) being tested positive OR 1.252[95% CI (0.096-0.426)] p = 0.213.

Table 4.4 Regression Analysis of Quality of sample and performance of SPANBIO

Characteristic	**(%)**	**OR (95% CI)**	***p* value**
Stool formed			
No	103 (70.1)	*ref.*	
Yes	44 (29.90)	1.06(0.856-1.069)	0.095
Semi formed			
No	94 (63.90)	*ref.*	
Yes	53 (36.1)	0.162(0.508-1.431)	0.871
Loose			
No	98 (66.70)	*ref.*	
Yes	49 (33.3)	0.814(0.659-1.275)	0.417
Fecal Pus cell			
No	116 (78.1)	*ref.*	
Yes	31 (21.9)	2.318(0.035-0.439)	**0.022**
Stool with mucus			
No	113 (76.90)	*ref.*	
Yes	34 (23.10)	2.982(0.089-0.440)	**0.003**
Stool color			
White	135 (91.80)	*ref.*	
Brown	12 (8.2)	1.077(0.475-1.026)	0.078
Stool with fat			
No	136 (92.5)	*Ref.*	
Yes	11 (7.50)	1.252(0.096-0.426)	0.213

Data presented are number and proportions (%) of subjects

CHAPTER FIVE

DISCUSSION

5.1 Demographic, clinical and laboratory characteristics of the study participants

Participant's age and gender were analyzed, these was an additional information that can add more information in the body of existing knowledge, and to guide policy makers on which age age group to tackle to reduce the burden of giardiasis. The mean age in cases versus controls were similar, however there was a significant difference in gender distribution between the participants who tested positive and participants who tested negative with more male among the participants who were positive to giardiasis than those who negative to giardiasis. These was comparable to similar cross-sectional study carried out in rural communities in Yemen where a total of 605 participants produced stool and tested for *G.lamblia*, and males accounted for 64.0% of the participants (Al-Mekhlafi *et al.,* 2017). This is further supported with another cross-sectional study done to 500 participants, in Sokoto, North-Western, Nigeria, that showed high prevelance of 85.4% and 14.6% intestinal infection in male and female, respectively (Mohammed *et al., 2015*). A school-based cross-sectional study done to 351 student in a primary school in Birbir town, Southern Ethiopia showed higher numbers of male pupils (180) and lower female numbers (171), having various or combinations of intestinal parasites (Alemu *et al., 2019*). Similarly, a cross-sectional study done to 364 participants, revealed, a prevalence of 51.4% of male verses 48.6% of female among participant who presented themselves at Shahura Health Center, North-west Ethiopia and were confirmed of having intestinal parasitic infection (Tibaju *et al., 2019*). These observations could be attributed to the higher exposure especially among men who have sex with men (MSM), this is a foreign cultures impacted to the local population (Espelage *et al.,* 2010). Males are also exposed to a wide range of infections, in African society males are the sole bread winner of the family, which may lead them to stay away from their families, thus exposing them to various risk factors, i.e., eating unhealthy and hygienically unsafe food, from unhygienic eateries.

Clinical evaluation did not show statistical difference in the distribution among participants who were; feeding poorly, bloating, vomiting or had flatulence, stomach-upset, and abdominal pain. There was significantly higher number of participants experiencing diarrhea among participants that were having positive results compared to those having negative results. In addition the number of foul smelling, stearic stool, mucoid, white colored stool, and the presence of fecal pus

cells were high. Despite the fact that the sign of fever, and symptoms of poor feeding, vomiting, flatulence, stomach up-set and abdominal pain, the higher numbers of headache, bloating, diarrhea, foul smelling stool, stearic stool ,mucoid stool, among participants testing positive, illustrated that these symptoms define giardiasis in this rural population. This study was able to capture several signs and symptoms, this was contrary to previous cross sectional study done in rural Malaysia where the study was able to reveal fewer symptoms (Choy *et al.,* 2014). From the study, 34.6% had diarrhea, while only 6.4% had abdominal pain, 10.3% had signs of vomiting, this results were somewhat similar for diarrhea and vomiting, with the current study, but the study had high numbers of participants with abdominal pains (Choy *et al.,* 2014). Again from another retrospective study carried out to 473 children, under 5 years presenting with acute gastroenteritis at a hospital in Northern Ghana showed 75% had both diarrhea and vomiting (Abdul-Mumin *et al., 2019*). Another cross-sectional study carried out on 1301 participants, aged between 22day -90 years, in Nahavand County, Western Iran, with intestinal parasite infection, showed that there was significant statistical difference in participants with diarrhea (37.8%) and constipation (12.9%) but no significance on abdominal pain, Nausea or vomiting, stomach pain, Bloating and Dysentery (Kiani *et al., 2016*). A cross-sectional study carried out on 500 children presenting with gastrointestinal disorders, in Nahavand County, Western Iran, revealed diarrheal stool (28.7%) was statistically significant, while abdominal pain, Nausea or vomiting, stomach pain, Bloating, and consistency of stool had no significance (Kiani *et al., 2016*).

The above mentioned symptoms have a systematic pattern of presentation, however there could be variations between participants. Therefore, these symptoms could be early signs of giardiasis during which it could be a localized acute inflammation, with production of Th2 (IL-4) followed by a shift toward a predominant Th2 (IL-5) response, likely associated with a counter regulatory mechanism of infection leading to production of Th1 (gamma interferon, interlukin-1beta, IL-6 and tumor necrosis factor, Th17 (IL-17), with intestinal secretory IgA and Systemic IgG in the gut (Serradell *et al.,* 2017). These cytokines promote inflammatory processes in the gastrointestinal system, leading to signs and symptoms such as mucus production, increased production of WBC leading to increased quantities of pus cells and ultimately affect test results of a particular device. The above information is vital in the diagnosis of gastro-intestinal infection as some of these signs and symptoms add information to an already existing knowledge in giardiasis. These may be more important in the screening and management of giardiasis.

These statistical difference could be as a result of various reasons. Recruiting mainly school going children as the subject can have tremendous effect on the study, another possible effect that can influence the outcome of a study is when the study was done i.e. season of the year which the study was performed, seasons come with varied climatic conditions, which can either propagate growth of this organism, or provide unfavorable condition for growth of this organism, therefore, causing increase or decrease of the causative agent in an environment, ultimately affecting the prevalence. Giardiasis is very common in rainy season and majority of these studies tend to take place during this period. Another likely possibility is social-cultural behavior, humans being are social animal and can propagate transmission of giardia from one person to another, that is, directly, for example, through sharing food from the same plate or pot in various ceremonies and even in our homes, to indirectly acquiring the infection through sexual activities, especially now when there is an increase of sexual activity within the same sex, these reasons can affect disease pattern, hence making a region good for a study. This current study was purely comparative cross sectional study. The results showing that males are the most affected group, is important as this will help the decision makers and the public health department to employ appropriate preventive measures that target this group.

5.1.1 Specificity of SPANBIO™ RDT compared to Microscopy

The present study demonstrated that relative to microscopy the specificity of SPANBIO™ RDT was 98.6% with a positive predictive value of 98.1% and accuracy of 81.6%, according to the aforesaid formula. These results are comparable to those from another retrospective and prospective study done in 12 hospital laboratories distributed across France, on a set of 482 stool samples, specificity was 99.3% on the retrospective study, with PPV of 97.1%, and 100% specificity when 339 from 482 samples were done prospectively (Goudal *et al.*, 2018).

However, another study involving children experiencing severe acute malnutrition (SAM) and diarrhea who were enrolled in a randomized controlled trial at clinical trials in one hospital in Blantyre, Malawi where the sample size was 175, and in two hospitals in Kilifi and Mombasa, Kenya, where the sample size was 120 and done between December 2014 and December 2015, revealed that Rida quick, Duo strip check had specificities of 92.1%, 95.5% and 98.0%, respectively (Bitilinyu-Bangoh *et al.*, 2019). A similar, comparative study done among 200 children, with microscopy as the gold standard, at the outpatient clinic of Assiut University Faculty children Hospital with complaints of diarrhea and other gastrointestinal symptoms,

showed RIDASCREEN having a specificity of 91.5% (Dyab *et al.,* 2016). Furthermore, a comparative study done on 96 stool specimen from patients who attended different primary health care centers in Al-Quma sector/Basrah health directorate, done between serazymELISA Giardia and RidaQuick Giardia with microscopy as the gold standard, showed specificity of 100% on serazymELISA and RidaQuick Giardia (Abdul-Mumin *et al.,* 2019). A similar, retrospective and prospective study where a total of 60 samples were tested, with ELISA and microscopy as reference and gold standard, respectively. Among the 60, 30 samples were frozen stool samples, for the retrospective study, and these samples were tested by ImmunocardSTAT, Crypto/Giardia Duo-Strip, RidaQuick and QuickCheck for *G.lamblia* and *Cryptosporidium*, these showed specificities of 100% to the four RDT's. The remaining 30 samples, for prospective study, were fresh stool and tested by Immunocard STAT, Crypto/Giardia Duo-Strip, RidaQuick and QuickCheck for *G.lamblia* and *Cryptosporidium* and specificities were 94% for the QuickCheck and 100% to the rest (Bossche *et al.,* 2015). A convenience study done on 388 felines in Colarodo, Oklahoma, and Virginia with commercially available kit in the United States with the direct immunofluorescent assay (IFA) as a reference standard showed that ZnSo4, Vet Chek, SNAP, VetScan and IFA had specificities of 98.6%, 99.7%, 97.2%, 99.3%, and 100% respectively (Saleh *et al.,* 2018).

A similar study involving 51 canine sample, tested at the Diagnostic Laboratories for copro-analysis using ZnSo4 and SNAP (concertation methods for microscopic stool analysis) as gold standards against single antigen Rapid Giardia Ag and the Triple Antigen Rapid CPV-CCV-Giardia Ag (BioNote) revealed specificity and PPV of 100% (Costa *et al.,* 2016). In addition, a study done among 82 adults patients attending Hermanos Ameijeiras clinical surgery hospital, comparing Paraset RDT with two microscopical technique, Richie's formalin ether and direct wet mount showed specificities of 100 % and PPV 100% (Yanet *et al.,* 2017). These high specificity, PPV, accuracy levels, from the current study, show that SPANBIO^{TM} RDT has high potential in the diagnosis of *G. lamblia* and this is further supported by kappa values of 0.6388 which is rated as 'good'.

This study had a total of three samples that had mixed infections, confirmed microscopically, that is, two stool samples had *Entamoeba hystolitica* cysts and , one had *Trichuria Trichuris* cyst, SPANBIO^{TM} RDT showed no Cross reactivity, studies have shown that specificity can be affected by cross-reactivity with other organism (Kheirabad *et al.,* 2017). The test kit/devices

mentioned above, from different studies, may have had different study design, to this current study, but they all employed test principles similar to that of SPANBIO^{TM} RDT, i.e., antibody/antigen reaction producing visible results. This kit is impregnated with a purified monoclonal antibody on the test band and giardin antigen on the control band. The test band only targets the giardin antigen thus significantly reducing chances of cross-reaction. Cross-reaction is common where the test device primarily targets antibodies from the test sample, and antibodies have variations on the variable region of the antibody structure, these variations are not always specific to a particular epitope of an antigen, because of their degree of purity, these variable regions can attach to various antigens with similar epitopes causing cross-reactivity, therefore, this aforesaid attribute of the SPANBIO^{TM} RDT likely and significantly reduces the probability of having a cross reaction phenomenal experienced with other antibody based test. Therefore, the results seen may not be true results, as these can come from non giardial species.

Specificity can also be affected by the following attributes which likely can affect the outcome of a study. First, when a study is being carried out in a place where there is low prevalence rate, the rate of giardia infection is partially dependent on certain prevailing conditions, which includes the climatic condition (mentioned earlier) giardiasis is more common in rainy season as this provide a moist condition, which is favorable for giardiasis to thrive. Secondly, state of immunity of the host, is a possibility that can increase or decrease prevalence of giardiasis, giardiasis is a self-limiting disease but in immuno-compromised individuals, like, HIV/AIDS patients, patients undergoing cancer treatment, children (<5), pregnant women and the elderly are highly susceptible of acquiring giardiasis, which can be life-threatening, these group generally have low immunity. Children, even as the innate and adaptive immune systems start to mature, is at risk from many pathogenic viruses, bacteria, fungi and parasites. The immune system gradually matures during infancy. First by the passive IgG antibody transferred from the mother transplacentally and in milk. Once that fades away, young children become more vulnerable to infections, though by then better armed with the maturing innate and adaptive immune systems. Pregnant women likewise have reduced immune levels because of the sharing of immune components with the unborn. As age advances, the immune system undergoes profound remodeling and decline, with major impact on health and survival. This immune senescence predisposes older adults to a higher risk of acute viral and bacterial infections. Moreover, the mortality rates of these infections are three times higher among elderly patients

compared with younger adult patients, this current study included individual of all age group. Therefore these groups of people can harbor and act as production center for *G.lamblia*, endangering others, and finally affecting the prevalence of giardiasis. Another possible factor that can also affect specificity is the type of study design, retrospective studies tend to produce specificities of up to 100%, these is possible as retrospective studies pool known and confirmed positive giardia samples and tested against a new test, resulting into high specificities, combining both retrospective and prospective study design therefore, can increase the specificities of the study as previously shown.

5.1.2 Sensitivity of SPANBIO™ RDT compared to Microscopy

In contrast, the sensitivity of SPANBIO™ RDT was low at 66.7%, positive predictive value was at 72.3 %, and these was comparable with another retrospective and prospective study done in 12 hospital laboratories distributed across France, on a set of 482 stool samples, showed a sensitivity of 89.2% on the retrospective study, with NPV of 97.3% (Goudal *et al.,* 2018). A similar, retrospective and prospective study where a total of 60 samples were tested, with ELISA and microscopy as reference and gold standard, respectively. Among the 60, 30 samples were frozen stool samples, for the retrospective study, and these samples were tested by ImmunocardSTAT, Crypto/Giardia Duo-Strip, RidaQuick and QuickCheck for *G.lamblia* and *Cryptosporidium,* these showed sensitivities of 58%, 83% 100% and 92%, respectively. The remaining 30 samples were fresh stool and tested by ImmunocardSTAT, Crypto/Giardia Duo-Strip, RidaQuick and QuickCheck for *G.lamblia* and *Cryptosporidium* and sensitivities of 79%, 66%, 83% and 100% (Bossche *et al.,* 2015). However another study involving children experiencing severe acute malnutrition (SAM) and diarrhea who were enrolled in a randomized controlled trial at clinical trials in one hospital in Blantyre ,Malawi where the sample size was 175 , and two hospitals in the coastal region, that is , one hospital in Kilifi and one in Mombasa, Kenya where the sample size was 120 and done between December 2014 and December 2015, revealed that Rida quick, Duo strip check had sensitivities of 52.9%, 52.9% and 82.4%, respectively (Bitilunyi-Bangoh *et al.,* 2019).

Another variant comparative study done among 200 children attending the outpatient clinic of Assiut University Faculty children Hospital with complaints of diarrhea and other gastrointestinal symptoms, showed RIDASCREEN Giardia test sensitivity was 100%. (Dyab *et*

al., 2016), Furthermore a comparative study done on 96 stool specimen from patients who attended different primary health care centers in Al-Quma sector/Basrah health directorate, done between serazymELISA Giardia and RidaQuick Giardia with microscopy as the gold standard, showed sensitivity of 90.1% on serazymELISA and sensitivity 79.0% on RidaQuick Giardia (Abdul-Mumin *et al.,* 2019). A convenience study done on 388 felines in Colarodo, Oklahoma, and Virginia with commercially available kit in the United States with the direct immunofluorescent assay (IFA) as a reference standard showed that ZnSo4, Vet Chek, SNAP, VetScan and IFA had sensitivities of 67.2%, 93.0%, 84.8%, 98.1%, and 100% respectively (Saleh *et al.,*2018). In addition, a study done to 82 adults patients attending Hermanos Ameijeiras clinical surgery hospital, comparing paraset RDT with two microscopical technique, Richie's formalin ether and direct wet mount showed sensitivities of 68.1 % and NPV 87.7% (Yanet *et al.,* 2017).

A Study involving 51 canine sample, tested at the Diagnostic Laboratories for coproanalysis using ZnSo4 and SNAP as gold standards against single antigen Rapid Giardia Ag and the Triple Antigen Rapid CPV-CCV-Giardia Ag (BioNote) revealed sensitivities of 48 and NPV of 83%, for the single tests and 55% sensitivity and 80% NPV for the triple tests (Costa *et al.,* 2016). Since giardiasis is a zoonotic disease and diagnostic methods used to detect this disease in animals are same to those done on humans, therefore, these makes studies from both man and animals to be of enormous value. Some of the documented substances that may obscure targeted antigen and ultimately lower the sensitivity are mucous, blood in stool, parasitemia density. From this study RDT recorded any parasite count below 3 as negative, and parasites between 3-7 count, was recorded as either positive or negative, these can be attributed to gene mutation or deletion, or the giardin gene had not reached detectable threshold in the sample. In a previous malaria study where malaria microscopy was compared with malaria RDT certain occurrence were observed, RDT was recorded negative but it was false negative, this occurs when parasitemia or parasite density is low (Wongsrichanalai *et al.,* 2007). Another possible factors that can have an effect on the sensitivity of RDT detection includes storage conditions of the gadget (Weitzel *et al*., 2006). Sensitivity may also be affected by Prozone effect (or high doses-hook phenomenon) that consist of false-negative or false-low results in immunological test, due to an excess of either antigen or antibodies (Carey *et al.,* 2016). This was never reported with SPANBIO^{TM}. This is a likely reason to why a test turn out as negative or occasionally invalid

test. From the 147 stool sample that were collected, one was microscopically negative but immuno-chromatographically positive by SPANBIO^{TM} RDT, this can be possible as a result of presence of giardin antigen in the guts in early stages of the disease, when there is either no or little shedding of the targeted antigen. Organisms are unique and exhibits different life-cycle, their uniqueness presents appreciated attributes that can either be detected phenotypically or genetically, by producing antigens or components that can be quantitatively or qualitatively analyzed through laboratory techniques that are applicable in the diagnostic field and eventually producing signs and symptoms of a particular medical condition.

Though diseases are different, caused by completely different species, the techniques used in their diagnosis may be the same, the difference may come about in specific immunological components used which targets a particular antigen or antibody, but the underlying test principle of these RDT kits is based on an antigen(Ag) and antibody(Ab) reaction, (Ag/Ab) reaction. Published malaria related documents, which used a test principle similar to that of SPANBIO^{TM} RDT, showed that foreign antigen takes time to be cleared from the human system, long after the infectious organism has been eliminated (Dairymple *et al.*, 2018). Therefore, a negative result does not exclude the presence of *Giardia lamblia* infection.

5.1.3 Sensitivity and Specificity of RDT compared to PCR

This current study illustrated that GDH PCR, the reference standard had a low sensitivity of 54.4% and a low specificity of 79.9%, with a positive predictive value of 69.8% and negative predictive value of 66.7% and accuracy levels of 67.0%, Cohen kappa was low (0.347) and, Mc Neymar's of 10.2. These results are similar to an evaluation study, done among healthy school going children aged 8-14 years in Centre-Ouest and Plateau Central regions, in Burkina Faso among 441 individuals, where sensitivity and specificity of PCR was 76.6 and 96.7%, respectively (Diagbouga *et al.,* 2017).

However, discrepancies was observed with a previous prospective study done to four hundred and twenty four patients, (227 males and 197 females), ranging in age from three (3) month to seventy (70) years attending outpatient department in Baquba general hospital, Iraq, this study showed high sensitivity of 94.8% and specificity of 98.0% of PCR (Al-Bayat *et al.,* 2015). These results may be as a result of the targeted gene in PCR, some genes are able to express themselves throughout the life cycle of *Giardia lamblia* from immature stage, the mature stage

and eventually the cystic stage. Since PCR is able to detect the targeted protein from early stage, makes it useful as a technique in validating SPANBIO^{TM} RDT. Again another cross sectional study, done in Bamako and Nioni, south central Mali, among 56 individuals, aged 2-63 years, that compared Coris duostrip RDT against PCR, showed a sensitivity of 62% for the RDT and 100% for PCR, while specificity was 94% for the RDT and 100% for the PCR (Fofana *et al.*, 2019). Another comparison study, between microscopy (gold standard) and PCR, done in the faculty of medicine, University Sebelas Maret, Indonesia, among 100 individuals having livestock, and live along the Bedog watershed, showed a sensitivity and specificity of 97.1% and 100%, respectively (Sari *et al.*, 2019). These high results could be possible as a result of the region on which this study was conducted, this is a region where there is expansive land for irrigation exposing the participants to schistosomiasis and giardiasis that mainly are dependent on moisture to flourish, and these conditions may highly increase the sensitivity and specificity of the results. This current study was in rural agricultural setting, that does not practice irrigation farming.

Furthermore a study done between August 2012 and April 2013 at Aarhus University Hospital, Denmark, where 455 samples were tested using salt/sugar floatation technique, microscopy of feces after formol-ethylacetate (FEA) and PCR on stool samples, with IFA as reference standard, showed PCR had high sensitivities and specificities of 91% and 95.1%, respectively (Gotfred-Rasmussen *et al.*, 2016). The process of PCR entails amplification of DNA picked from fecal samples and amplified into many copies, which can be detectable by electrophoresis, and since giardiasis, depending on the immunity, can be self-limiting, these means it can clear itself, leaving destructed particles in the gut. Clearance of this organism can be accomplished by either white blood cell, mainly easonophile, which can produce toxic substances that are capable of destroying the organism, or by immunoglobulins (IgG) and compliment components (C3 and C5) causing opsonisation eventually resulting to death of the organism, with the end product being DNA or proteins remnants, from the destroyed parasite present in the body circulation. These remnants may take time for them to be entirely removed or cleared from the system, eventually giving a positive results by PCR, therefore there is a likely-hood of the sensitivity of PCR being high because of this reason. Certain medicines and medicinal products can also destroy or cause death of giardia eventually releasing genetic and phenotypic components of giardia in the digestive system, and later on affecting sensitivity of PCR. Therefore, there is a

likelihood of a patient presenting all the cardinal signs and symptoms of giardiasis but on microscopic examination and immuno-chronographically by RDT the patient test negative.

5.2 Factors associated with SPANBIO™ RDT positivity

SPANBIO™ RDT reactivity among the study participant did not show difference in the distribution among participants who were feeding poorly, had flatulence, stomach-upset, abdominal pain. There were significantly higher number of participants experiencing diarrhea among the cases compared to those in control group, in addition the rates of foul smelling, stearic stool, mucoid, white colored stool (signifying a possible fatty stool sample), or the presence of fecal pus cells were higher in cases relative to controls.

Although symptoms of poor feeding, vomiting, flatulence, stomach up-set and abdominal pain were similar between cases and control, the higher numbers of participants experiencing headache, bloating, diarrhea, foul smelling stool, stearic stool, mucoid stool, among cases illustrated that this symptoms could significantly influence the test results. During the manifestation of the disease, certain chemical are naturally produced by the body, such as a neurotransmitter called serotonin, this is a chemical produced majorly in the guts, when someone is experiencing headache, (Young, 2007), and varied amount of gases like methane and sulfide, when the human body experience bloating, foul smelly stool and presence of pus cells, blood (RBC), cytokines, and antibodies in diarrheic stool (Serradell *et al*., 2017). There is a possibility of these substances altering or interfering with test performance of a kit as they can alter molecular weight colloidal gold particles. Many RDT use the principle of immuno-chromatography where they are impregnated with a mixture of colloidal gold and specific antibody on the test band while the control band has only the colloidal gold. Upon exposure to certain substances, the molecular weight and density changes leading to visible color change, when there is a reaction, (Koczula *et al.*, 2016). Chemical and substances produced during the process of infection have a like-hood of altering test performance. Gas production in the guts, get dissolved in stool changing the pH level of stool, pus cell which can lead to the masking of the targeted antigen, blood which has high content of iron that are reducing agent, and various ions and proteins including cytokines and antibodies produced during diarrhea. From this study, appearance of these substances can possibly and significantly affect test performance. Previous medical review, have shown that substances like proteins, ions like potassium, sodium affects biochemical parameters in blood (Cuhadar, 2013). However, there is no previous documented or

published study that can be comparable or illustrating how these substances affected test performance on a rapid diagnostic technique. Macroscopic stool features occurring at high rates (foul smelling stool, stearic, stool with presence of pus cells and loose stool) suggest that these stool characteristics may affect result of SPANBIO^{TM} RDT. A study conducted in Asembo, Siaya County, Kenya on children showed that diarrhea is highly associated with intestinal protozoans (Miranda *et al.,* 2018). But a cohort study done from 1291dog fecal samples showed there is no significant association between loose stool and Giardia infection in the overall population (Uiterwijk *et al.,* 2019).

Further regression analysis of the aforementioned characteristics, that significantly affected test performance, showed that, fecal pus and mucus greatly affected test performance of SPANBIO^{TM} RDT. Fecal pus are dead white blood cells, their death maybe be associated with ageing or infection, significant amount of fecal pus cells signifies an impending infection or medical condition, white blood cell are part of the body's immune system (Seladi-Schulman, 2018). Conversely, mucus are majorly composed of water, epithelial cells, dead leucocytes (pus cells), mucin (heavily glycosylated proteins), and inorganic salts (Mestecky et al., 2015). These substances have high impact to the performance of Spanbio^{TM} RDT, it could possibly be as a result of their capability of masking the targeted protein of *Giardia lamblia*. Consistency of stool is important, as it acts as a guide on what the microscopist expect in fecal sample. This study showed that formed stool significantly affected test performance of SPANBIO^{TM} RDT, there is a likely-hood of *Giardia lamblia* to be found in semi-formed or loose stool but rarely can *G.lamblia* found in formed stool.

CHAPTER SIX

CONCLUSIONS, RECOMMENDATIONS AND RECOMMENDATION FOR FUTURE STUDIES

6.1 Summary

SPANBIO^{TM} RDT had high specificity and positive predictive value with low sensitivity and negative predictive value with an agreement considered to be good when compared to PCR and microscopy. Specificity and sensitivity results of RDT were comparable to those of PCR. Stool with high proportions of mucus and fecal pus interfered with the performance of the test.

6.2 Conclusions

i. The specificity and positive predictive value of SPANBIO^{TM} RDT compared to microscopy, was high at 98.6%) and (98.1%), respectively, and there was test performance agreement between the two tests.

ii. The sensitivity and negative predictive value of SPANBIO^{TM} RDT compared to microscopy was low at (66.7%) and (72.3%), respectively,

iii. Mucus and fecal pus in stool highly influenced the test performance of SPANBIO^{TM} RDT, this was after a regression analysis was done.

6.3 Recommendations from the current study

i. The Given a high specificity of SPANBIO^{TM} , the technique can be recommended to be used in a resource limited rural health facility to diagnose patients suspected of having giardiasis, by laboratory personnel, clinicians and even nurses.

ii. The low sensitivity indicate that this test should be used with precise signs and symptoms, mostly by clinician and nurses, in out-patient department, in areas with high prevalence of giardiasis.

iii. Key stool characteristic should be considered while selecting and performing the test by laboratory personnel as these can be valuable in the diagnosis of *G. lamblia* using SPANBIO^{TM} RDT.

6.4 Recommendation for Future Studies

i. Further studies should be done to determine whether other intestinal organism have antigenic composition similar to that of *G.lamblia* and effect on Specificity.

ii. Further molecular test should be done to determine why *G. lamblia* organisms produce varied amounts of various proteins and the approximate population in the environment and effect on sensitivity.

iii. Further studies should be done to compare SPANBIO^{TM} RDT with other immunological techniques. Also further study should focus on how to improve results by use of gastric juice that may contain the organism and minimal inhibitants like mucus and pus.

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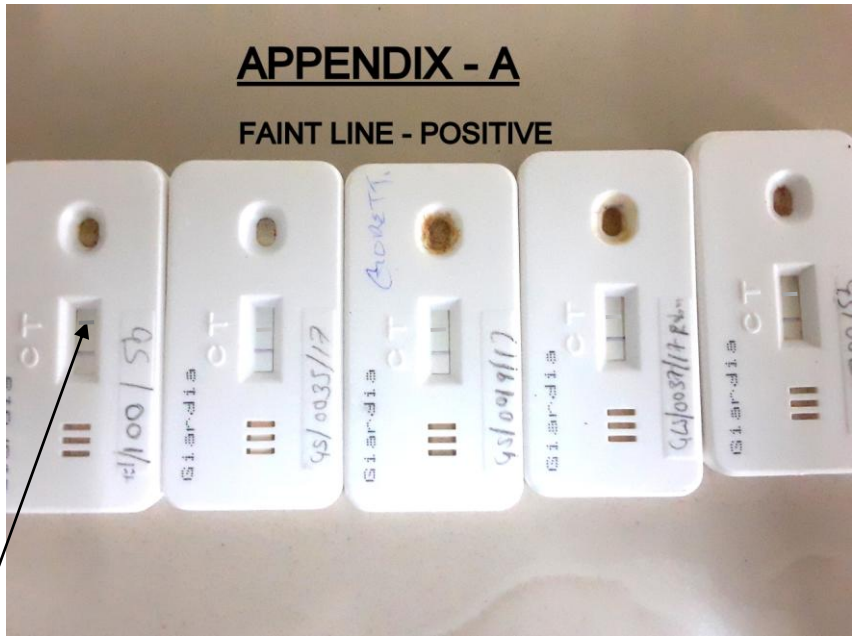
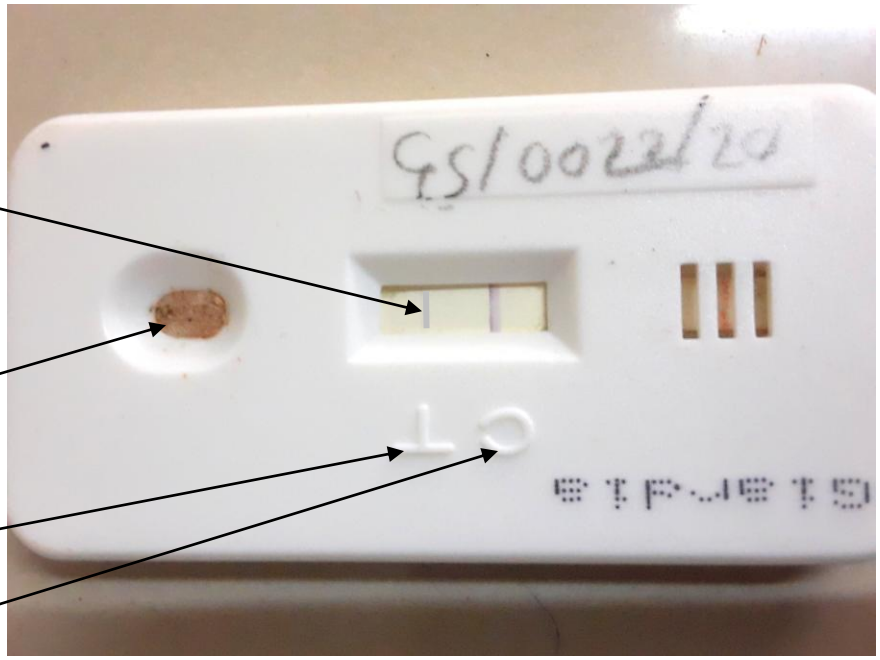
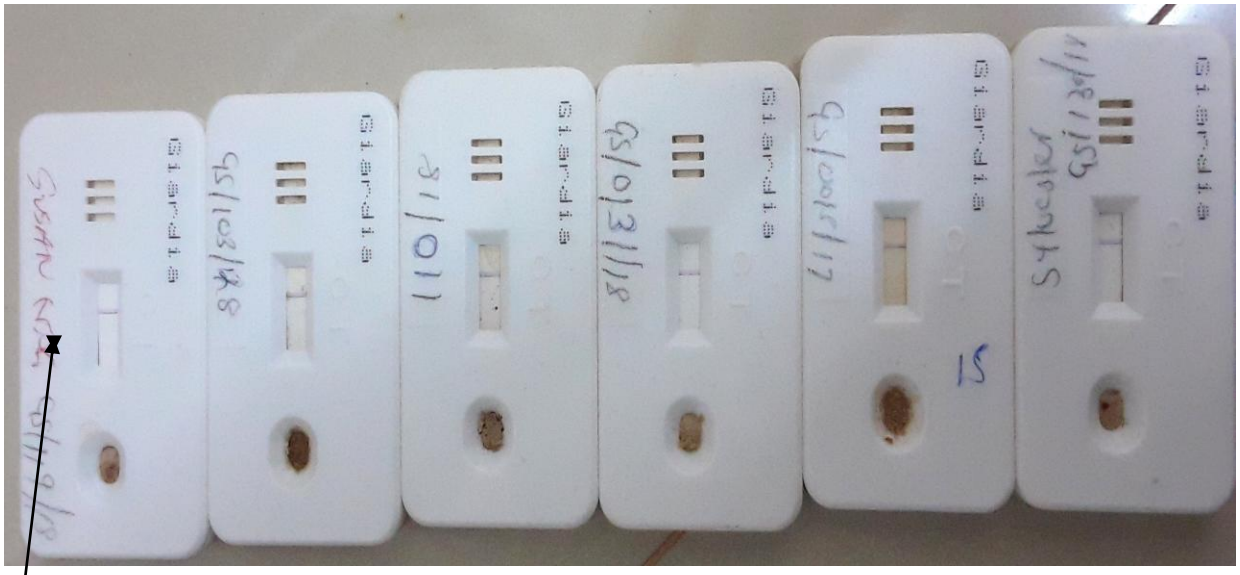
APPENDICES

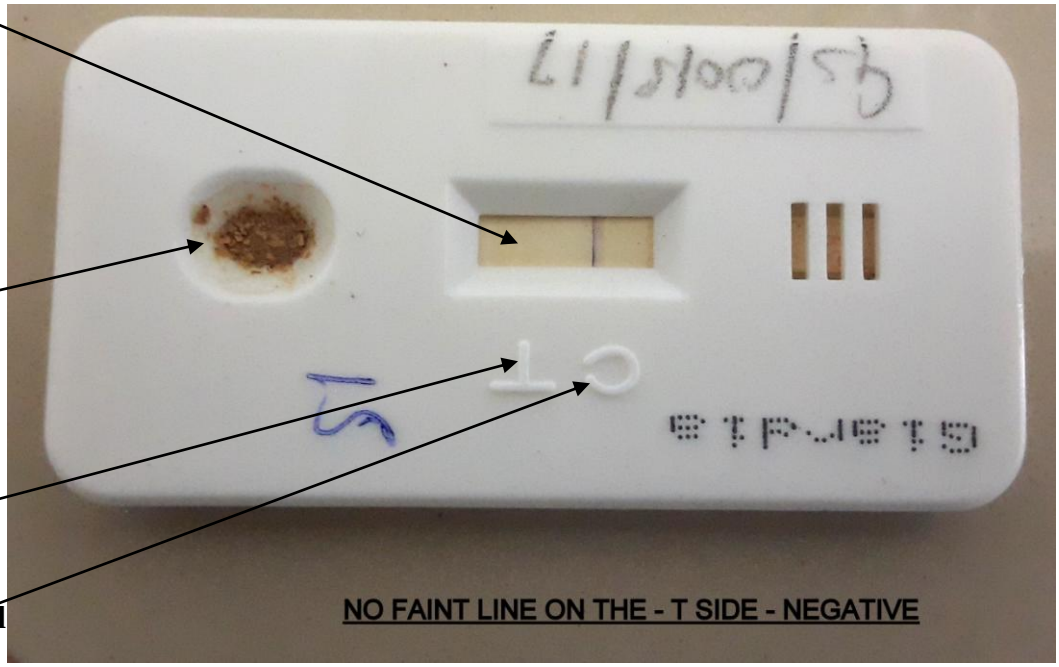
Appendix A: Faint Line Positive





Appendix B: No Faint Line On The T(Test) Side Negative





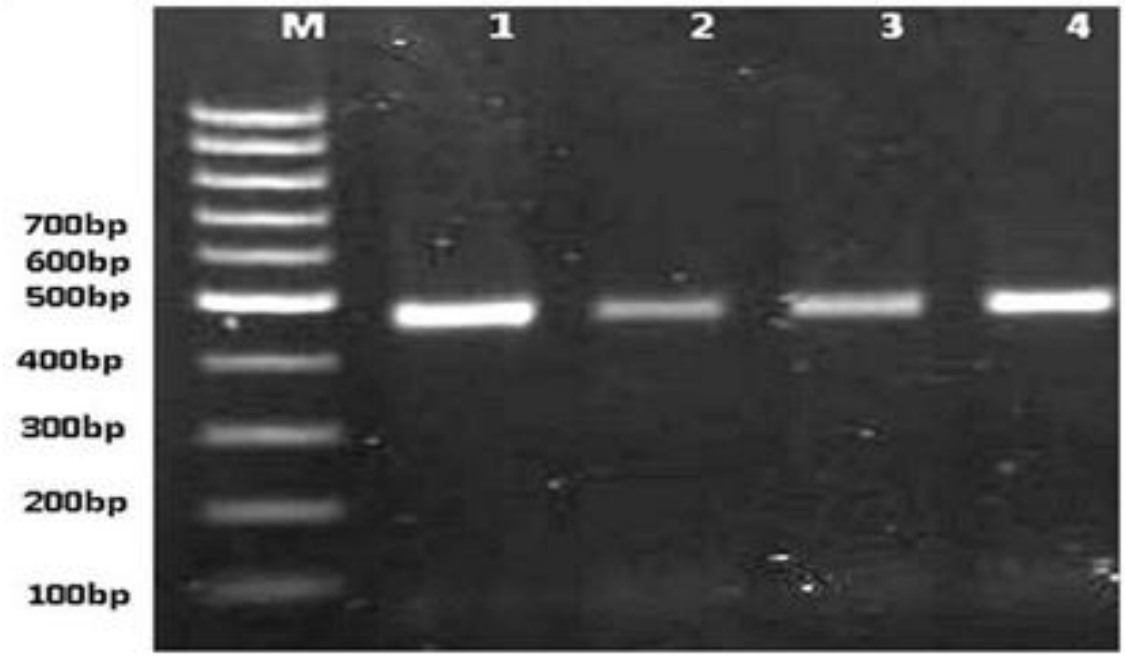
No faint line

Test sample

Test band

Control band

Appendix C: PCR



Representative PCR for gdh gene amplification 532 bp

Appendix D: Data Collection Approval

DEPARTMENT OF HEALTH AND SANITATION
OFFICE OF THE MEDICAL SUPERINTENDENT

Telegram: 'MEDICAL', Busia
Telephone: 055 22126, /22136
Fax: 05522136
E-mail: busiahospital@gmail.com
When replying please quote

OFFICE OF THE MEDICAL SUPERINTENDENT
BUSIA COUNTY REFERRAL HOSPITAL
P. O. BOX 87
BUSIA (K)

Date: 21st September, 2017

BSA/CRH/R/5V0L.11/92

BRISTON ROBERTS INDIEKA,
P.O.Box836,
LUANDA.

Dear Sir,

RE: AUTHORITY TO CONDUCT RESEARCH ON PERFORMANCE OF ONE –STEP RDT IN DETECTION OF *GIARDIA LAMBLIA* IN BCRH.

Your request to conduct research at Busia County Referral Hospital on the above subject has been accepted.

You are therefore allowed to start your research on 25th September 2017 to 15th August, 2018. At the completion of your research kindly share your research findings with the Administration for identification of possible improvements remedies.

Thank you,

Yours faithfully,

MEDICAL SUPERINTENDENT
24 SEP 2017
P. O. Box 87-50400 BUSIA(K)

CHARLES MAKOKHA
FOR: MEDICAL SUPERINTENDENT
BUSIA COUNTY REFERRAL HOSPITAL.

Appendix E: Ethical Approval

MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221

Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 20th September, 2017

TO: Briston Roberts Indieka
PG/MSc/PH/0098/2013
Department of Biomedical Science and Technology
School of Public Health and Community Development
Maseno University
P. O. Box, Private Bag, Maseno, Kenya

REF: MSU/DRPI/MUERC/00367/17

RE: Evaluating the Performance of One-Step Test in Detection of *Giardia lamblia* in Busia County Referral Hospital, Western Kenya. Proposal Reference Number MSU/DRPI/MUERC/00367/17

This is to inform you that the Maseno University Ethics Review Committee (MUERC) determined that the ethics issues raised at the initial review were adequately addressed in the revised proposal. Consequently, the study is granted approval for implementation effective this 20th day of September, 2017 for a period of one (1) year.

Please note that authorization to conduct this study will automatically expire on 19th September, 2018. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 15th August, 2018.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 15th August, 2018.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

Thank you.

DIRECTORATE OF RESEARCH, PUBLICATION & CONSULTANCIES
20 SEP 2017
MASENO UNIVERSITY

Dr. Bonuke Anyona,
Secretary,
Maseno University Ethics Review Committee

Cc: Chairman,
Maseno University Ethics Review Committee.

Appendix F: Approval Letter For The County Referral Hospital

BRISTON R INDIEKA,
P.O.BOX 836,
LUANDA.
10TH MAY 2016

THE DIRECTOR OF HEALTH,
BUSIA COUNTY,
P.O.BOX …..
BUSIA.

Dear Sir/Madam,

RE: REQUEST FOR PERMISSION TO CONDUCT RESEARCH IN BUSIA COUNTY.

My name is Briston Indieka, an MSc student at the Maseno University, Department of Biomedical Sciences. I wish to conduct a research study for thesis writing in fulfilment of the MSc program. The title of the thesis research is '**Evaluating the Performance of one-step rapid test in detection of *Giardia lamblia* in Busia County Western Kenya'**. This research project will be conducted under the supervision of Dr Tom Were (Department of Medical Laboratory Sciences, Masinde Muliro University of Science and Technology) and Dr Benard Guya (Maseno University).

I hereby seek your consent to conduct the research in Busia County referral hospital so as to recruit participants for this research.

I have provided you with a copy of my abstract, which includes copies of consent and assent forms to be used in the research process, as well as a copy of the ethical approval letter which I received from the Maseno University Research Ethics Committee.

Upon completion of the study, I undertake to provide the Department of Health with a bound copy of the full research report. If you require any further information, please do not hesitate to contact me on 0723544809 and email address brindieka@yahoo.com.

Thank you for your time and consideration in this matter.

Yours faithfully,

Briston. R. Indieka

Appendix G : Giardiasis Case Questionnaire

Date of the interview…….....Name of the interviewee…….

Name of the interviewer……Relation of the case……..

Part A: Demographic information

First name……..Last name……...

Date of birth:../…../……. (Mm/dd/yyyy) Age (yrs.)…… Sex……

Height……... (m) Weight……. (Kg)

Waist circumference…… (cm) Hip circumference…….. (cm)

Physical address……Sub County……

Highest level of education……

State type of house……..

State number of people in that house hold……...

Unique number …….

Occupation ……...

Guardian's occupation……..

Approximate monthly income (Ksh)<5000……..>5…….

Date last attended work/ daycare/ school (please specify Day school/boarding): _____ (mm/dd/yyyy)

*High risk occupations are food handlers, health care workers, child care workers, children in child care, and residents of institutions.

SECTION II: TREATMENT INFORMATION

Did you visit a clinician (Medical officer, Clinical officer, Nurse) (circle one) Yes No

Name of clinic or hospital: _____

Were you admitted to hospital? Yes No Date of admit? _____ (mm/dd/yyyy)

Did you receive treatment? Yes No Name of treatment? _____

Date treatment started :_____(mm/dd/yyyy) Date treatment completed :_____(mm/dd/yyyy)

SECTION III: SYMPTOM INFORMATION

Date and time of onset: _____ (mm/dd/yyyy) am pm

Symptoms:

Fever Yes No Unknown

Nausea Yes No Unknown

Vomiting Yes No Unknown

Abdominal pain Yes No Unknown

Headache Yes No Unknown

Diarrhea Yes No Unknown

Was diarrhea watery / bloody/greasy? _____

Duration of illness: _____ (hours/ days)

Comments:_____

_____

_____

SECTION IV: CONTACT INFORMATION

In the two weeks prior to illness, has the case had contact with a family member/ friend/ colleague with similar illness?

Yes No Unknown

	Name of contact	Telephone number	Physical address

SECTION V: ENVIRONMENTAL RISK FACTORS

In the two weeks prior to illness did any of the following risk factors apply?

Travel Yes No If yes, travel where? _____

Dates of travel: _____ (mm/dd/yyyy) to _____ (mm/dd/yyyy)

Close contact with farm animals (including petting zoos): Yes No

Location: _____ Date of visit: _____ (mm/dd/yyyy)

Any pets (including fish and reptiles) at home? Yes No Specify:

_____

Water source piped or well, if others please specify _____

Toilet type Flush toilet/pit latrine/bush, others please specify

Drinking source water: Boiled/purified water others please specify

Do you garden? Yes No If yes, contact with potting mix or manure? Yes No

Specify brand of potting mix or/ and manure:

_____

Did you participate in swimming or water sports? Yes No

If yes please specify……..

When was the last time you ate out please specify (hotel, restaurant, kiosk, roadside food)

Comments:_____

_____

_____

_____

Have you had alcohol Yes No?

How long ago……...

How much……..

Appendix H: Laboratory Request Form

Name.....…...DOB…….Sex…….

Surname/First/Middle name dd/mm/yy

Physical address……..

Unique no…….

Department (Tick where applicable)

Parasitology…….Hematology……...

Clinical chemistry……...Bacteriology……...

Blood transfusion ……Histology……..

History……

……

……

……..

Requisiting officer……..Signature……..

Results……

……

……...

Please specify consistency for examination of stool (Watery, Loose, Formed, Bloody, Greasy)

Examined by……Signature……..…….

Approved by……..Signature……