



## A Comparative Study of Microscopy and Rapid Antigen Test for the Diagnosing Protozoan Diarrhea in Cancer Patients in Wasit Province, Iraq

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### Abstract

**Background:** Intestinal protozoal infections, particularly those caused by *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum*, pose serious health risks for immunocompromised individuals. Cancer patients are especially vulnerable, as these infections often lead to severe diarrhea and other gastrointestinal complications. **Methods:** Stool samples were examined microscopically using direct smear and modified Ziehl–Neelsen staining. In addition, a rapid antigen test was performed to detect antigens of *E. histolytica*, *G. lamblia*, and *C. parvum*. **Results:** The overall detection rate of intestinal protozoal infections was significantly higher using the rapid antigen test (53.3%, 48/90) compared with microscopy (18.9%, 17/90). Based on the rapid test results, *G. lamblia* accounted for 43.75% of positive cases, *C. parvum* for 37.5%, and *E. histolytica* for 18.75%. The highest infection rates were observed among patients aged 41–56 and 57–72 years. A statistically significant association was found between age group and infection rate with both diagnostic methods (Microscopy:  $p = 0.041$ ; RAT:  $p = 0.003$ ). Participants were evenly distributed among breast, gastrointestinal, and hematological malignancies (33.3%) each. Participants were evenly distributed among breast, gastrointestinal, and hematological malignancies (33.3%) each. **Conclusion:** The rapid antigen test demonstrated greater sensitivity than microscopy for detecting intestinal protozoal infections in cancer patients. The high prevalence observed highlights the need for regular screening of this immunocompromised population. For rapid diagnosis and improved patient management, it is strongly recommended that rapid antigen testing be integrated into standard diagnostic protocols.

**Keywords:** Intestinal protozoa, Cancer Patients, Rapid antigen test, Microscopy

دراسة مقارنة بين الفحص المجهرى واختبار المستضد السريع لتشخيص الإسهال الناتج عن الأوليات لدى مرضى السرطان في محافظة واسط، العراق

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### المستخلص

الخلفية: تُشكل العدوى المعوية بالطفيليات الأولية، وخاصةً تلك التي تُسببها الأميبا الحالة للنسج (*Entamoeba histolytica*) والجيارديا اللمبية (*Giardia lamblia*) والكريبتوسبورديوم الصغير (*Cryptosporidium parvum*)، مخاطر صحية جسيمة للأفراد ذوي المناعة الضعيفة. ويُعد مرضى السرطان أكثر عرضةً للخطر، إذ غالبًا ما تؤدي هذه العدوى إلى إسهال حاد ومضاعفات معوية أخرى. الطرق: تم فحص عينات البراز مجهرًا باستخدام المسحة المباشرة وصبغة زيل-نيلسن المُعدلة. بالإضافة إلى ذلك، أُجري اختبار سريع للكشف عن مستضدات الأميبا الحالة للنسج والجيارديا اللمبية والكريبتوسبورديوم الصغير. النتائج: كان معدل الكشف الإجمالي عن العدوى المعوية بالطفيليات الأولية أعلى بكثير باستخدام اختبار الكشف السريع (53.3%، 48/90) مقارنةً بالفحص المجهرى (18.9%، 17/90). استنادًا إلى نتائج الاختبار السريع، شكلت الجيارديا اللمبية 43.75% من الحالات الإيجابية، والكريبتوسبورديوم بارفوم 37.5%، والأميبا الحالة للنسج 18.75%. سُجلت أعلى معدلات الإصابة بين المرضى الذين تتراوح أعمارهم بين 41 و56 عامًا، وبين 57 و72 عامًا. وُجد ارتباط ذو دلالة إحصائية بين الفئة العمرية ومعدل الإصابة باستخدام كلتا طريقتي التشخيص (الفحص المجهرى:  $p = 0.041$ ؛ اختبار المستضد السريع:  $p = 0.003$ ). توزع المشاركون بالتساوي بين أورام الثدي، والجهاز الهضمي، والدم (33.3% لكل منها). الخلاصة: أظهر اختبار المستضد السريع حساسية أعلى من الفحص

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معلومات البحث

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المجهري في الكشف عن عدوى الأوليات المعوية لدى مرضى السرطان. يُبرز الانتشار المرتفع الملاحظ ضرورة إجراء فحص دوري لهذه الفئة من المرضى الذين يعانون من نقص المناعة. من أجل التشخيص السريع وتحسين إدارة المرضى، يوصى بشدة بدمج اختبار المستضد السريع في بروتوكولات التشخيص القياسية.

**الكلمات المفتاحية:** الأوليات المعوية، مرضى السرطان، اختبار المستضد السريع، المجهري

## Introduction

Intestinal parasites are a major public health issue, particularly in tropical and subtropical regions. About 3.5 billion people are infected, and most of them are children in poor countries. Protozoa and helminths are two types of intestinal parasites that infect humans. *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium species* are the most common protozoa that infect humans [1]. There are two main forms of intestinal parasites: helminths and protozoa. Protozoa are single-celled organisms that belong to the Protista kingdom. They can multiply in the human body, which can lead to serious infections [2]. When cells grow too quickly and can invade other cells, that's when cancer happens. Also, the number of people with cancer has been going up a lot in the last several years [3]. Parasitic diseases are still common and pose a hazard to human health. As society has grown, so have the environment and way of life. Along with better traffic and more people being able to move around, the number of cases of parasitic disease is also changing. Additionally, the population with immunodeficiency or weakened immunity is growing due to more organ transplants, more cases of cancer and AIDS, and higher usage of immunosuppressors. Thus, opportunistic parasites are becoming more and more significant as diseases. Changes in natural conditions also affect where and how many parasites there are [4]. The immunocompromised host is typically characterized as a person who has experienced one or more deficiencies in the standard defensive

systems that protect against infections, hence elevating the individual's susceptibility to severe, life-threatening infections [5]. In Iraq, gastrointestinal parasite infections are the primary causes of mortality and morbidity, resulting in several public health challenges, including malnutrition, anemia, and growth retardation [6]. People can obtain intestinal parasite diseases directly or indirectly by eating or drinking infected food, water, fruits, or vegetables, or by touching objects contaminated with fecal matter, like fingers [7;8]. There are three types of diarrhea: acute (lasting less than 2 weeks), persistent (lasting more than 2 weeks but less than 4 weeks), and chronic (lasting more than 4 weeks). It is quite hard to figure out what causes chronic diarrhea and parasite illnesses [9]. The comparison between microscopic inspection and antigen strip tests is performed to ascertain which diagnostic approach offers superior accuracy, sensitivity, and reliability in detecting intestinal parasite infections. Microscopy is often thought of as the standard touchreference method, but it has some big problems. For example, it relies on the examiner's skill, the results can be hard to interpret, and it has limited sensitivity when there aren't many parasites or when they are shed only occasionally. Antigen strip tests, on the other hand, use immunological detection of parasite-specific antigens. This makes them more standardised, provides faster results, and lets you find infections that might not show up under a microscope. Thus, a comparative assessment of these two

methodologies is essential to guarantee accurate diagnosis, improve early detection in both clinical and subclinical instances, and inform the choice of the most successful diagnostic strategy in both routine and epidemiological contexts [10]. Parasitic infection of the intestinal tract is a significant etiology of several clinical manifestations, including diarrhea, anorexia, flatulence, weight loss, abdominal discomfort, nausea, vomiting, and fever, as well as intestinal obstruction [11]. The parasites possess numerous antigens due to the complexity of the host's bodily structure, enabling them to dodge the host's immune response through several mechanisms, including alteration of their surface antigens, encystment, and migration [12]. Microscopic inspection is still the most common way to find intestinal parasite diseases. It involves looking directly at cysts, trophozoites, oocysts, or eggs in stool samples. It is cheap and easy to find, but its accuracy depends a lot on the examiner's skill, the quality of the sample, and the stage of the illness.

On the other hand, immunochromatographic strip tests (also called rapid antigen tests) for antigen detection are faster and more accurate since they find parasite-specific antigens straight from stool samples. These tests are easy to use, don't require much technical skill, and can find infections even when there aren't enough parasites for a microscope to see. This makes them very useful for routine screening and in the field [13]. The laboratory diagnosis of intestinal parasite infections mostly relies on microscopic examination of stool samples to identify helminth eggs and protozoan trophozoites and cysts. Certain parasite species cannot be distinguished only through microscopy, whilst the detection of others (*E. histolytica*, *G. lamblia*, and *Cryptosporidium spp.*) may require highly skilled experts [14]. The

etiological diagnosis is based on the microscopic identification of cysts and trophozoites in the patients' feces, together with the detection of certain antibodies and antigens [15]. Immunological procedures, rapid detection techniques such as immunochromatographic strip tests and direct methods using PCR techniques, and histological examination are further ways to diagnose [16].

The aim of the present study was to determine the prevalence of intestinal protozoan infections among cancer patients at Wasit Province.

## Materials and Methods

### Collection of stool samples

Stool samples from patients at Wasit Specialized Center for Cancer Treatment were collected, during the period from August 2024 to February 2025. About 90 stool samples were collected. A questionnaire was completed that covered kind of infection from patients and age, place of residence, sex, and duration, as well as sterilized plastic containers with a tight lid that is used to retain stool samples to prevent dryness and moisture. stool

### Stool sample examination

#### Macroscopic examination

The volume of feces, form, consistency, and color of stool volume, consistency, shape, and color were evaluated during examination. Trophozoites frequently appear in liquid or soft specimens, whereas cyst phases can be found in semisoft samples. The sections of the feces that may contain blood or mucus should be carefully checked as it may contain the parasite's trophozoites stage. The liquid sample and mucus should be analyzed first if there were multiple samples brought to the

laboratory.

### Microscopic examination

The examined stool samples with direct smear methods involved putting a tiny drop of normal saline (0.9%) or iodine stain on the sliding glass, mixing well with a tiny amount of stool using a wooden stick, covering the slide, and looking at the sample under 40× and 100×power magnification Staining by hot modified Ziehl–Neelsen stain, this method is used for parasites such as *Cryptosporidium parvum*.

### Method of Rapid test (Chromatographic immunoassay)

**CerTest Crypto + Giardia + Entamoeba combo card test** offers a simple and highly sensitive screening assay to make a presumptive diagnosis of cryptosporidiosis and/or giardiasis and/or amoebiasis.

### Manual Procedure:

The stool samples and controls were kept at room temperature and checked for dispersion. Before using the combo card test, a small amount of feces was collected and dispensed into the circular window marked with *E. histolytica*, *G. lamblia*, and *C. parvum*. If the result window has two lines (T red, C green), the kit is working and positive results are obtained. Results were read after 10 minutes.

### Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the

Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol, subject information, and consent form were reviewed and approved by a local ethics committee, according to document number 1509, dated August,5,2024, to get this approval.

### Statistical analysis

Statistical analysis was performed using the SPSS version 28.0. Data were subjected to normality test. The normally distributed data are expressed as mean  $\pm$  standard deviation and compared with one way analysis of variance (ANOVA) among three groups. Data with abnormal distribution were compared with the non-parametric test. Qualitative data are expressed as number or percentage, and rates were compared using the  $\chi^2$  test. A value of  $P < 0.05$  was considered statistically significant.

### Results

The result of the study were obtained from the analysis of 90 Cancer Patients who were recruited in this study. Table (1) shows the Age group (years). The highest frequency was observed in the (41-56) year age group, with a frequency of (30) , i.e., a percentage of (33.3%), and the lowest frequency of Age is (9-24), with a frequency of (4), i.e., a percentage of (4.4%). and shows the number and percentage of each type of cancer, as the numbers and percentages were equal for all types (Gastrointestinal, Breast and Hematology), i.e., with a number of (30) patients for each type and a percentage of (33.3) for each of the three .

**Table (1): Age Distribution of the Study Sample (N=90)**

Age group (years)	Frequency	Percent
9-24	4	4.4
25-40	22	24.5
41-56	30	33.3
57-72	23	25.6
73-88	11	12.2
<b>Total</b>	<b>90</b>	<b>100</b>

Table (2) shows a frequency distribution table and percentages for the gender variable, as the number of males was (36) at a rate of (40%) and the

number of females was (54) at a rate of (60%) of the sample under study.

**Table (2): sex Distribution of the Study Sample (N=90)th**

Sex		Frequency	Valid Percent	Cumulative Percent
Valid	Male	36	40.0	40.0
	Female	54	60.0	100.0
	<b>Total</b>	<b>90</b>	<b>100.0</b>	

Table (3) shows the number and percentage of each type of cancer, as the numbers and percentages were equal for all types

(Gastrointestinal, Breast and Hematology), i.e., with a number of (30) patients for each type and a percentage of (33.3) for each of the three.

**Table (3): Frequency and Distribution of Cancer Types in the Study Sample (N=90)**

Type of cancer		Frequency	Valid Percent	Cumulative Percent
Valid	Gastrointestinal	30	33.3	33.3
	Breast	30	33.3	66.7
	Hematology	30	33.3	100.0
	<b>Total</b>	<b>90</b>	<b>100.0</b>	

Table (4) The association of age group with the infections per parasite for Microscope:For Microscope it was noted that the highest frequency is (3) and for the percentage (17.65%) For age

group (41-56) with *C. parvum* and last place with a frequency of (0) For age groups (9-24)and (73-88) with *G. lamblia* and *E. histolytica*, there was

significant association (P-value=0.041) chi-square test criterion value (7.937).

**Table (4) Association Between Age Groups and Prevalence of Intestinal Protozoan Infections by Microscope**

Age group (years)	Microscope No. (%)			Total	P-Value
	<i>C. parvum</i>	<i>G. lamblia</i>	<i>E. histolytica</i>		
9-24	1 (5.88%)	0 (0 %)	0 (0 %)	1 (5.88%)	<b>0.041</b>
25-40	1 (5.88%)	2 (11.76%)	1 (5.88%)	4 (23.5%)	
41-56	3 (17.65%)	2 (11.76%)	2 (11.76%)	7 (41.18%)	
57-72	1 (5.88%)	2 (11.76%)	1 (5.88%)	4 (23.5%)	
73-88	1 (5.88%)	0 (0 %)	0 (0 %)	1 (5.88%)	
<b>Total</b>	7 (41.18%)	6 (35.29%)	4 (23.5%)	17 (100%)	
<b>χ<sup>2</sup> Value</b>	<b>7.937</b>				

Table (5) For **ANTIGEN** it was noted that the highest frequency is (7) and for the percentage (14.58%) For age group (57-72) with *Cryptosporidium parvum* and last place with a

frequency of (0) For age groups (9-24) and (73-88) with *Entamoeba histolytica*, there was significant association (P-value=0.003) chi-square test criterion value (7.045).

**Table (5): Association between Age Groups and Prevalence of Intestinal Protozoan Infections by ANTIGEN**

Age group (years)	ANTIGEN No. (%)			Total	P-Value
	<i>C. parvum</i>	<i>G. lamblia</i>	<i>E. histolytica</i>		
9-24	1 (2.08%)	2 (4.17%)	0 (0 %)	3 (6.25%)	<b>0.003</b>
25-40	2 (4.17%)	5 (10.42%)	3 (6.25%)	10 (20.83%)	
41-56	5 (10.42%)	8 (16.7%)	3 (6.25%)	16 (33.33%)	
57-72	7 (14.58%)	5 (10.42%)	3 (6.25%)	15 (31.25%)	
73-88	3 (6.25%)	1 (2.08%)	0 (0 %)	4 (8.33%)	
<b>Total</b>	18 (37.5%)	21 (43.75%)	9 (18.75%)	48 (100%)	
<b>χ<sup>2</sup> Value</b>	<b>7.045</b>				

**DISCUSSION**

The total sample size in this study was 90 stool samples collected from cancer patients at Wasit Specialized Center for Cancer Treatment between August 2024 and February 2025. This study has several limitations that should be considered when

interpreting the findings. First, the relatively small sample size of 90 cancer patients from a single center in Wasit Province may limit the generalizability of the results to other regions or oncology populations with different demographic and clinical characteristics. Second, the cross-

sectional design and reliance on stool-based microscopy and a single rapid antigen test mean that intermittent shedding of protozoa or low parasite burdens could still lead to underestimation of true prevalence, despite the higher sensitivity of the rapid test compared with conventional microscopy. This study has several limitations that should be considered when interpreting the findings. First, the relatively small sample size of 90 cancer patients from a single center in Wasit Province may limit the generalizability of the results to other regions or oncology populations with different demographic and clinical characteristics. Second, the cross-sectional design and reliance on stool-based microscopy and a single rapid antigen test mean that intermittent shedding of protozoa or low parasite burdens could still lead to underestimation of true prevalence, despite the higher sensitivity of the rapid test compared with conventional microscopy. The present study aimed to determine the prevalence of intestinal protozoan infections, specifically *E. histolytica*, *G. lamblia*, and *C. parvum*, among cancer patients in Wasit Province, Iraq, and to compare the diagnostic efficacy of conventional microscopy versus a rapid antigen detection test (RAT). The key findings indicate a significant prevalence of these protozoan parasites in the studied immunocompromised cohort. Microscopy identified infections in 17 out of 90 patients (18.9%), while the more sensitive RAT detected antigens in 48 out of 90 patients (53.3%). This substantial discrepancy underscores a critical limitation of microscopy, particularly in a patient population where high-sensitivity diagnosis is paramount. In table (4) intestinal protozoan infections showed a statistically significant association with age group ( $\chi^2 = 7.937$ ,  $p = 0.041$ ). The highest proportion of cases was observed

among participants aged 41–56 years (41.18%), whereas markedly fewer infections were detected in the youngest (9–24 years) and oldest (73–88 years) age groups. This pattern may reflect age-related differences in exposure to contaminated food or water, occupational and behavioral risk factors, and underlying health or immune status in middle-aged adults. This finding is consistent with a study conducted at Al-Najaf University of Alkafeel 387 stool samples were examined, and 97 samples were positive for testing for intestinal parasites showed that the most affected age group is 41-56 years in the table(4)[17]. This finding is consistent with a study conducted in Kut city A total of 50 feces samples were collected .The results appeared that 15 (30%) of feces samples were positive for *E. histolytica* examined by microscope 40X who showed trophozoite and cyst[18]This study is agree with study in Sohag University, Egypt [19]. This finding is consistent with a study conducted in Basrah A total of 106 fecal specimens were collected were found to excrete Cryptosporidium oocyst in their stool, compared to the 31.1 % of patients with malignant diseases during chemotherapy. *Giardia*, 8.5% of patients during chemotherapy [20]. This study is agree with study in Zanjan University of Medical Sciences, Zanjan, Iran. 100 fecal samples were collected from patients with cancer the findings in Iran that the most common parasite among cancer patients was *C. parvume* followed by *G. lamblia* [21]. This study is agree with study in Sohag University, Egypt the most common parasite among cancer patients was *C. parvume* followed by *G. lamblia* followed *E. histolytica*[22]. On the other hand in Thi-Qar Province disagreed The total number was 260 cancer Patients. The total number of a positive patients for intestinal parasite *C. parvum* were 2(2.85%) [23]. This study is

inconsistent with a study conducted in Thi-Qar (200) stool samples from cancer patients shows that the rate of infection with parasite Oocysts depends on the age group, as it was noted that the highest infection rate was in the age group  $\geq 61$ , followed by the age group 31-40, and the lowest infection rate in the age group  $\leq 20$ . The infection rates among them, respectively, were as follows: 64.44%, 56.66%, and 35.71%. The statistical analysis showed significant differences between them ( $p < 0.05$ ) [24]. In table(5) The present findings demonstrate a significant relationship between age and the occurrence of intestinal parasitic infections ( $P = 0.003$ ). Infections were most common among adults aged 41–56 and 57–72 years, whereas markedly lower rates were noted in the younger (9–24 years) and older (73–88 years) age groups. This distribution suggests that exposure risk may be influenced by lifestyle and occupational factors. Adults in the middle age range are typically more active in the community and workplace, which may increase contact with contaminated food, water, or environments. In contrast, younger individuals may benefit from greater parental supervision and improved hygiene practices, while elderly individuals might have reduced exposure due to limited mobility or dietary restrictions. This study is agree with study in Saudi Arabia 148 stool samples examined rapid immunoassay, 20 *G. lamblia* positives and 13 for *C. parvum* [25]. The higher detection rate by RAT is consistent with global literature.

Immunochromatographic tests are designed to detect specific parasite antigens (e.g., *G. lamblia* CWP1 protein, *E. histolytica* Gal/GalNAc lectin, *Cryptosporidium* spp. antigens) with high specificity and superior sensitivity compared to microscopy, especially in cases of low parasite burden or intermittent shedding [26;27]. This is

especially important for people with cancer who are on treatments that lower the number of parasites but not the chance of complications, which can make microscopy less reliable [28]. The prevalence of parasites found by RATG—*Giardia lamblia* (43.75%), *Cryptosporidium parvum* (37.5%), and *Entamoeba histolytica* (18.75%)—corresponds with research indicating that *Giardia* and *Cryptosporidium* are especially common and troublesome in immunocompromised persons, leading to severe, chronic diarrheal disease [29]. Another important finding is the link between age and the number of infections. Microscopy and RAT both found that the age ranges 41-56 and 57-72 had the highest incidence of infection. This may be linked to the higher rates of several cancers in these age groups, as well as maybe higher exposure to the environment or caregiver situations that make transmission more likely. The very low percentage in the youngest (9–24) and oldest (73–88) groups may be due to the fact that these groups had fewer people in them or that they had different types of cancer and treatment plans. The even spread of cancer kinds (Gastrointestinal, Breast, Hematological) in our sample lets us compare parasite risk more closely. It is commonly known that people with blood cancers (such leukemias and lymphomas) and people who are getting hematopoietic stem cell transplants are among of the most immunocompromised. They have serious problems with both cell-mediated and humoral immunity, which makes them very likely to have opportunistic protozoan infections like cryptosporidiosis, which can cause cholangiopathy and diarrhea that lasts for a long time [30]. In the same way, people with gastrointestinal cancer may have weak gut integrity, which makes it easier for parasites to get in and makes their nutritional condition worse [12]. The high prevalence

identified in this study shows that people in these high-risk groups need to be checked for parasites on a regular basis. The higher performance of RAT in this investigation validates the developing paradigm in parasitological diagnosis. Microscopy is still the best way to find a wide range of parasites and check how active trophozoites are moving, but it has some problems. For example, it depends on the operator, is not very sensitive, and can't tell the difference between some parasites (like *E. histolytica* and non-pathogenic *E. dispar*) [31]. RAT is a quick, easy-to-use, and very accurate point-of-care technique for the particular and important diagnosis of the three protozoa in this investigation. This is very important in a clinical oncology environment since a quick diagnosis can directly affect how a patient is treated, such starting the right antiprotozoal therapy (for example, nitazoxanide for cryptosporidiosis) and taking steps to stop infections from spreading [32].

### Conclusions

In conclusion, our study shows that a lot of cancer patients in Wasit Province have intestinal protozoan infections. Rapid antigen testing are far better at finding these infections than traditional microscopy. Cancer patients are particularly susceptible to severe complications from these infections because of their compromised immune status.

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