

# Comparative Evaluation Study of Rk39 Dipstick and On-Site Leishmaniasis Testing for the Retrospective Diagnosis of Visceral Leishmaniasis in Misan Governorate, Iraq

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**Annotation:** This retrospective study evaluated diagnostic accuracy of the rK39 strip test and the On-Site leishmaniasis technique for the identification of visceral leishmaniasis (VL) in Misan Governorate, Iraq. VL, caused by various *Leishmania* strains, used to require invasive diagnostic procedures. This study aimed to determine the impact of non-invasive serological testing procedures, specifically the IgG/IgM combined in situ leishmaniasis test (ISLT), introduced in Iraq in 2017, as well as the eK39 dipstick assay, known for its parameters rate (sensitivity and specificity). Human serum samples were collected and tested using both methods. The results indicate a correlation between the two tests, suggesting their suitability to aid in diagnosis in the examined samples. The two diagnostic tools did not show any statistically significant differences ( $P>0.05$ ). These findings contribute to our understanding of visceral leishmaniasis diagnostic methods and their applicability

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in Iraq, indicating that both the on-site IgG/IgM visceral leishmaniasis test and the rK39 strip test provide reliable, non-invasive alternatives for diagnosing this disease.

**Keywords:** *Leishmania donovani*, visceral leishmaniasis diagnosis, rK39 strip tests, visceral leishmaniasis test, visceral leishmaniasis diagnosis, K39 antigen.

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## Introduction:

Different species and strains of *Leishmania* parasites are associated with visceral leishmaniasis. (Kirch, 2008). However only minority individuals with confirmed infection by *Leishmania* develop advanced visceral leishmaniasis, defined by symptoms such as pyrexia, hepatosplenomegaly, anemia, neutropenia, in addition to hypergammaglobulinemia, the majority of infected individuals may show no symptoms (asymptomatic) ,so the disease often resolves on its own (Berman,1997and Kumar, 2021 ).

The frequency of visceral leishmaniasis (VL) in Iraq is highest during the winter and spring months, with an increase in cases observed from December to March (CDC, 2005). According to the Report on Leishmaniasis in Iraq for the first quarter, Ministry of Health, Communicable Diseases Control Center, the disease exhibits seasonal patterns (p. 1-2).

the visceral leishmaniasis diagnosis has depended on invasive methods but non-invasive serological methods have been developed (Cota et al., 2013). However, these tests require specialized equipment. The direct agglutination test (DAT) offers a more accessible alternative but necessitates analysis at distant medical centers. The using of PCR amplification to detect *Leishmania* DNA is not feasible under field conditions. (Sundar et al.,1998 and Cota et al., 2013).

In 2017, a new rapid test, the OnSite™ *Leishmania* IgG/IgM Combo test (CTK Biotech, USA) (Freire et al., 2018), was introduced inside Iraq. This test is intended for identify *Leishmania*-specific IgG and IgM antibodies in human serum samples; however, there is currently no data on its utility in identifying VL cases caused by *Leishmania infantum*. This study assessed effectiveness of the OnSite™ *Leishmania* IgG/IgM Combo test (Freire et al., 2018) in identifying Visceral Leishmaniasis inside Iraq, notwithstanding its previous purchase and distribution within the public health system.

rK39 band test detects antibodies to the rK39 antigen in human blood or plasma samples. The rK39 band test uses an antigen produced as resulting from infection with *Leishmania donovani* , the cause of visceral leishmaniasis (VL). High sensitivity & specificity make this test reliable for detecting VL ( Zijlstra et al., 2001). It delivers quick and accessible outcomes in a brief period. The existence of antibodies targeting the rK39 antigen signifies a current or recent infection with *Leishmania donovani*. The rK39 strip test has proven its effectiveness as a significant instrument for diagnosing and tracking VL in regions where it is common (Badaró et al., 1996; Singh et al., 2009; Maia et al., 2012).

The current retrospective study seeks to assess the efficacy of these two diagnostic tests for VL in Misan Governorate, Iraq. Human serum samples were gathered and examined utilizing the OnSite *Leishmania* IgG/IgM Combo test (Freire et al., 2018) and rK39 strip . Outcomes were evaluated and compared to determine utility of these assays in diagnosing visceral leishmaniasis. This study's main aim is to evaluate the agreement and precision of the OnSite *Leishmania* IgG/IgM

Combo test (Freire et al., 2018) and rK39 strip assay in identifying visceral leishmaniasis in Misan Governorate, Iraq.

Our research seeks to enhance the guidelines for improved diagnostic tools for visceral leishmaniasis and healthcare methods in Iraq, while also providing significant insights into the application of non-invasive serological tools for diagnosing the disease.

### **Materials and methodology**

The method for gathering human serum samples was as follows:

1. Gather two milliliters of blood from each patient.
2. Separate the serum from the red cell clot promptly to avoid hemolysis.
3. Perform the test immediately after serum collection.
4. Allow the serum to reach room temperature before starting the test.
5. Take out the Kalazar Detect test for VL from its packaging.
6. Add 20  $\mu\text{L}$  of serum to the designated area on the test strip.
7. Position the test strip in a test tube or well tissue culture plate according to the indicated arrows on the strip.
8. Place 2 or 3 drops (approximately 150  $\mu\text{L}$ ) provided chase buffer on test strip.

**Diagnostic tools employed in this study to detect Leishmania infection were the rK39 strip test and the OnSite™ Leishmania IgG/IgM Combo test (Freire et al., 2018)** , Regarding rK39 strip test, procedures were conducted following the manufacturer's protocols. The test involves a test strip membrane that contains IgG is detected by an immobilised anti-protein-A antibody at the top and a band of rK39 antigen at the bottom. The immunochromatographic detection reagent is a protein A-gold conjugate. Three drops of test buffer are added to the absorbent pad at the bottom of the strip after a drop of serum. Through capillary action, mixture migrates up strip, and after 10 minutes, a red upper line appears as a control, confirming the presence of IgG and proper test functioning. A red lower line indicates the presence of anti-rK39 IgG and signifies a positive test result. The test strip membrane is manufactured by InBios, in Seattle, WA, USA.

**The OnSite™ Leishmania IgG/IgM Combo test (Freire et al., 2018) distinguishes itself from other rapid tests as it has the capability to detect both IgG and IgM antibodies. the protocol for the OnSite™ Leishmania IgG/IgM Combo test (Freire et al., 2018) :**

1. Ensure test kit is at room temperature.
2. Remove the tester from the sealed bag and place it on a clean, stable work area.
3. Optionally, the test device can be labeled with the patient's identifying information.
4. Add 5  $\mu\text{L}$  of the patient's serum or plasma sample into the test device of the sample well (S) using disposable pipette or dropper.
5. Place two or three (80-120  $\mu\text{L}$ ) to provided sample buffer into same sample well (S).
6. Allow the test to develop and wait for colored lines to appear.
7. After 15 to 20 minutes, read the results and make sense of them. Don't try to figure out what the results mean after 30 minutes. If only one red line, which is the control line, shows up, the manufacturer says the test is negative. If the M line, which shows the presence of IgM antibodies, or the G line, which shows the presence of IgG antibodies, or both lines appear along with the control line, it is a positive result. The test has been made to take into account all possible band reactivity, which means it looks at all possible reactions.

## Results

The study was conducted at Maysan Children and Maternity Hospital, and it was a retrospective study in the year 2021. The study aimed to compare two types of kits used for diagnosing leishmaniasis in suspected children admitted to the hospital. The kits used were the Onsit kit and the rK39 Test. Figure (1,2,3,4)

**Table 1. Diagnosis of Visceral Leishmaniasis in Misan Governorate, Iraq, in 2021 using the rK39 strip test and The OnSite™ test**

Positive leishmaniasis for rK39	%	Negative leishmaniasis for rK39	%	The total	
				Number of suspected having visceral leishmaniasis	Percentage of infected with leishmaniasis
5	8.3	55	91.7	60	8.3
positive leishmaniasis for The OnSite™	%	Negative leishmaniasis The OnSite™	%	Number of suspected having visceral leishmaniasis	Percentage of infected with leishmaniasis
9	15	51	85	60	15

This table provides information on visceral leishmaniasis diagnosis in Misan Governorate for year 2021 using rK39 Test and the OnSite™ test. According to the rK39 Test, the total percentage of individuals infected with leishmaniasis is 8.3%, with 55 suspected cases (91.7) testing negative and 5 cases (8.3%) testing positive. In comparison, the OnSite™ test shows a higher infection rate, with a total percentage of 15% infected individuals. Among the 60 suspected cases, 85% tested negative (51 cases) and 15% tested positive (9 cases) for leishmaniasis using the OnSite™ test. There are no significant differences at the significance level of  $P \leq 0.05$  when using the Chi-Square test.

**The table 2 shows the distribution of visceral leishmaniasis according to gender using the rK39 test and the Onsit kit.**

Test	Male	%	Female	%	The total	
					Number	%
rK39	4	6.7	1	1.7	5	8.3
Onsit	6	10	3	5	9	15

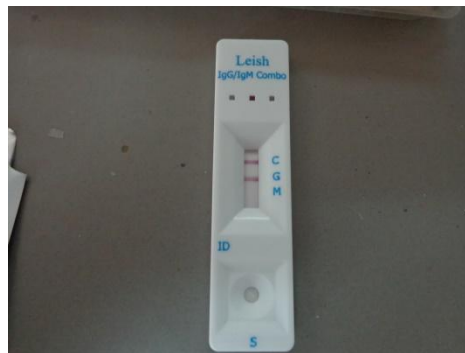
This table provides information on the distribution of visceral leishmaniasis based on gender using the rK39 test and the Onsit kit. According to the rK39 test, the total percentage of infected individuals is 8.3%, with 1.7 % being females (1 case) and 6.7% being males (4 cases). In comparison, the Onsit kit shows a total infection rate of 15%, with 3 cases (5%) being females and 6 cases (10 %) being males. There are no significant differences at the significance level of  $P \leq 0.05$ .

**The table 3 presents the diagnosis of visceral leishmaniasis using the Onsit kit compared to the use of the rK39 immunochromatographic strip.**

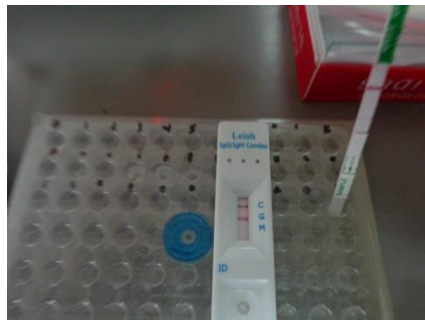
The sequence		Onsit	rK39
1	January	+	+
2	January	+	-
3	January	+	-
4	February	+	-

5	March	+	+
6	March	+	-
7	April	+	+
8	April	+	-
9	May	+	+
The total		9	5

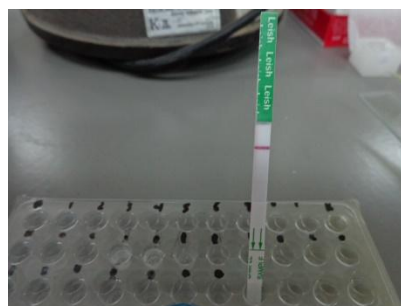
This table presents the results of diagnosing visceral leishmaniasis using the Onsit kit compared to the rK39 immunochromatographic strip. In January, the result was positive in the Onsit test, while it was negative in the rK39 test. In March, April, and May, the results were positive in the Onsit test, while they were negative in the rK39 test. In total, there were nine positive cases in the IgG test and five positive cases in the Onsit test. There are no significant differences at the significance level of  $P \leq 0.05$  when using the Chi-Square test.



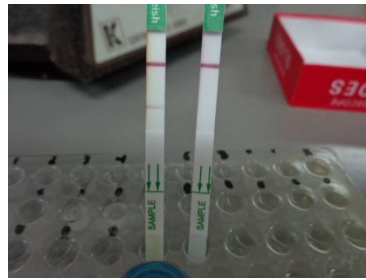
**Figure (1) IgG Positive= Late stage or latent infection.using Onsit kit**



**Figure (2) IgG positive = late stage or latent infection using Onsit kit (left),positive infection using rK39 immunochromatographic strip.**



**Figure (3) negative infection using rK39 immunochromatographic strip.**



**Figure (4) positive infection using rK39 immunochromatographic strip.(left),negative infection (right )**

## Discussion

This study demonstrates that both the K39 strip test and the Onsit test yield reliable and sensitive results in detecting active kala-azar. These tests are particularly suitable for field conditions as they require minimal peripheral blood samples and do not necessitate sophisticated laboratory equipment. Moreover, the test procedures themselves are simple to perform and interpret. In situations where many families are unwilling to subject their child to bone marrow aspiration, we can rely on these tests for diagnosing Kalazar.

The study reveals that substituting established diagnostic procedures with a rapid and straightforward test for a single antigen poses a substantial risk in accurately diagnosing patients with fatal visceral leishmaniasis. While the rapid strip test exhibits high specificity, as supported by previous studies, it currently lacks the necessary sensitivity to be recommended as a primary screening tool. Nonetheless, it can still play a valuable role in guiding further diagnostic procedures for suspicious cases and can serve as a confirmatory test during clinical examinations.

Given the absence of a standardized diagnostic method for identifying asymptomatic infections, utilizing a combination of various techniques to ascertain their actual prevalence currently represents most favorable approach (Ibarra-Meneses et al., 2017; Le Fichoux et al., 1999).

When comparing the positive rates of infection using both types, it is attributed to the fact that each strip is used for diagnosing a different type of *Leishmania*, the rK39 test is used to diagnose VL caused by the species *Leishmania infantum*, which prevalent in Misan Governorate, most regions of Iraq, and the Eastern Mediterranean region. On the other hand, the OnSite™ test is used to diagnose VL caused by pathogen species *Leishmania donovani*.

Many Cases have been reported of *Leishmania donovani* infections in Basra province, but they account for a small percentage, reaching up to 14%, of the visceral leishmaniasis cases in Basra. These cases are primarily associated with work and trade activities in previous years., due to migration and displacement of Basra residents to other provinces such as Baghdad, Thi-Qar, Najaf, Karbala, and Muthanna due to wars, there have been some cases of *Leishmania donovani* infections, accounting for 8% of the infections in these provinces. The remaining percentage of infections is caused by *Leishmania infantum*.

however, the current study indicating that the there is no significant differences between them at  $P \leq 0.05$ .

it good to mention that there is limitation of this study. this study design is retrospective, which inherently introduces the possibility of selection bias. The samples used may not entirely capture the entire population of Misan Governorate, and choice of samples could impact the observed diagnostic performance of the tests.

moreover, while we aimed to assess the performance of these diagnostic tests, we did not explore the clinical outcomes and treatment responses of patients who tested positive or negative. A comprehensive evaluation of these aspects would provide a more holistic understanding of the diagnostic methods' clinical utility.

To build on this research, coming studies could adopt a prospective design that involves a larger and more diverse patient population. This could help healthcare providers optimize their diagnostic strategies based on the time of year. Incorporating clinical follow-up data into future studies would also enable researchers to assess the clinical implications of positive and negative test results. The information is fundamental for guiding treatment decisions.

### Conclusion:

This retrospective investigate highlights diagnosis on visceral leishmaniasis in Misan Governorate, Iraq. The combined on-site visceral leishmaniasis IgG/IgM test and the rK39 cassette test appear to be effective and easy-to-use alternatives to more invasive methods. The study confirms their efficacy, while emphasizing the need for future research and attention to clinical outcomes. This study significantly improves the diagnosis of leishmaniasis, particularly in areas with varying incidence rates, potentially leading to improved early detection and improved patient outcomes. This marks an important advance in the field of leishmaniasis diagnosis.

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