Performance of Rapid Rk39 Tests for the Diagnosis of Visceral Leishmaniasis in Ethiopia: A Systematic Review and Meta Analysis

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Abstract

Background

Visceral Leishmaniasis (VL) is a severely neglected disease affecting millions of people with high mortality if left untreated. In Ethiopia, the primary laboratory diagnosis of VL is by using rK39-based rapid diagnostic tests (RDT). Different RDT brands are available with very variable performance and studies from Ethiopia showed a very wide range of sensitivity and specificity. Therefore, the objective of this systematic review and meta-analysis was to determine the pooled sensitivity and specificity of rk39 RDT in Ethiopia.

Method:

PUBMED, EMBASE, and google scholar were searched using predefined search terms to systematically retrieve all relevant articles. Eligibility screening was independently performed by two members and disparities were solved by other reviewers in the team. Methodological quality was also checked using the quality assessment of diagnostic accuracy studies (QUADAS-2) checklists.

Results

A total of 664 articles were retrieved from the PUBMED, EMBASE and google scholar online database searches. After removal of duplicates and initial, and final selection steps, 12 articles were included in the study. The overall pooled sensitivity and specificity of all rk39-based RDTs reported to diagnose VL in Ethiopia were 88.0 % (95%CI: 86.0–89.0%) and 84.0 % (95% CI: 82.0–86.0%), respectively. The sensitivity and specificity of the two rk39-based commercial test kits were for DiaMed RDT: 86.9% (95% CI: 84.3–89.1%) and 82.2 % (95% CI: 79.3–85.0%), and for InBios RDT: 80.0% (95% CI: 77.0–82.8%) and 97.4% (95% CI: 95.0–98.8%), respectively.

Conclusion

The sensitivity and specificity of rk39-based RDTs for the diagnosis of VL in Ethiopia were found to be lower compared to other parts of the world. However, the sensitivity of rk39-based RDTs was higher than in other east African countries. On the other hand, the specificity was lower than the east Africa studies (91.1%). The sensitivity and specificity of the rk39-based RDTs were in northern Ethiopia notably lower than in the other parts of Ethiopia.

Background

Visceral leishmaniasis (VL), or kala-azar, is a neglected tropical parasitic disease caused by a group of intracellular hemoflagellate protozoans of the genus *Leishmania*, and transmitted via the bite of infected female *Phlebotomine* sandflies[1, 2]. Over 90% of the global VL burden is attributed to six less developed countries: Bangladesh, Brazil, India, Ethiopia, Sudan, and South Sudan [3–5]. Ethiopia ranks third among the world most VL affected countries and around 3.2 million people in the country are at risk of contracting the disease [6, 7]. The northern and northwest parts of the country has the highest-burden, (Fig. 1), which accounts for nearly 30–40% of the total number of Ethiopian VL patients [8]. It is estimated that about 30% of the VL patients are also malnourished and co-infected with HIV, especially in the northern region of Ethiopia [9].

As VL is a deadly disease, timely and accurate diagnosis is important to install appropriate treatment [5]. The diagnosis is based on the combination of clinical signs and symptoms with laboratory confirmation [10]. The laboratory confirmation is done by demonstrating *Leishmania* parasites in microscopic preparations from splenic, or bone marrow, or lymph node aspirates, which is considered to be the gold standard test [11]. However, the low sensitivity combined with the invasive and risky sample collection procedures deterred the implementation of microscopy in the remote endemic areas of Ethiopia [12]. To circumvent the drawbacks of direct parasitological methods, serology has now been put in place in many regions of the country for the diagnosis of VL[13]. The direct agglutination test (DAT) is a pioneer serological test based on the agglutination of a *Leishmania* promastigote antigen preparation with specific antibodies in patient serum, which result can be interpreted without any reading aid. The DAT is robust, as the freeze-dried antigen with proven high sensitivity and specificity in all VL endemic regions around the world at an affordable price[14]. The drawback of DAT is the relative long overnight incubation and RDTs have been proposed as alternatives. In particular Rk39-based tests that detect antibodies against the 39-amino acid repeat antigens encoded by a kinesin-related gene of the amastigotes stage of the *Leishmania infantum* [15, 16], is considered to be a good alternative. RK39-based RDTs are simple to perform, cost-effective, stable at room temperature, and rapid. These immunochromatographic tests are currently widely implemented for the diagnosis of VL in resource-limited countries like Ethiopia [17, 18]. However, limitations of rk39-based RDTs are variable specificity, inability to differentiate between current and past infection, not being suitable for treatment effectiveness monitoring [19].

Studies performed in Ethiopia, which evaluated the diagnostic accuracy of rk39 RDT, showed a large variation with sensitivities, ranging from 27.8% [20] to 98.3% [21]. Similarly, the specificities also showed a huge variation from 27.8% [20] to 98.2% [22]. Despite these variations, Ethiopia does not have nationwide and regional data that showed the diagnostic accuracy of the rk39 test. Therefore, this review and meta-analysis aim to determine the pooled national sensitivity and specificity of the rk39 test and to assess if there is a difference between the different regions of the country.

Method And Materials

Study Design

A systematic review and meta-analysis was performed following the Cochrane library recommendations for determining diagnostic test accuracy to assess the nationwide pooled sensitivity and specificity of the rk39-based RDTs produced by InBios International Inc. (Seattle, WA, USA) or DiaMed-IT Leish®, DiaMed AG, Cressiersur-Morat, Switzerland, DiaMed Bio-RAD France, and Kalazar Detect® (InBios International, USA, and onsite Leishmania Ab Rapid Test (CTK Biotech, USA). Articles that determined the diagnostic performance of rk39-based RDTs for the diagnosis of VL in Ethiopia were retrieved from MEDLINE, EMBASE, and Google scholar databases.

Inclusion criteria

Original articles that determined the diagnostic accuracy of the index tests (rk39) for diagnosis of VL using human specimen, have a reference/s test, the actual number of true positive, true negative, false-positive, and false-negative presented and has a clear classification of study subjects into VL patients and controls were included in the systematic review and meta-analysis.

Exclusion Criteria

Articles were excluded if not clearly define the reference test and patient and control groups. Studies that used non-human specimens, were also excluded.

Search strategy

Duplicates cleaning on the basis of titles was performed by using *EndNote X8* software. Primarily selection was done by reading titles and abstracts. Subsequently, articles found eligible by initial selection were further screened by reading the full-text. The initial screening was independently performed by Dawit G. Hagos and Dr. Dawit Wolday. Discrepancies were resolved by discussion among the members of the team. To retrieve the relevant articles, the following search terms were included: PUBMED:- *(((((("leishmaniasis visceral"[MeSH Terms] OR "Kala-azar"[tiab]) AND "L. infantum"[tiab]) OR "L. donovani"[tiab]) OR "L. chagasi"[tiab]) AND (("diagnostic performance"[tiab] OR "rk39" [tiab]) OR "validation"[tiab])) AND "Ethiopia"[tiab]*. The search terms for EMBASE were:- *(('visceral leishmaniasis'/exp OR 'kala azar'/exp OR 'l. donovani' OR 'l. infantum') AND 'performance of rk39' OR 'evaluation of rk39' OR 'evaluation'/exp) AND Ethiopia.* The quality of the selected articles was evaluated using QUADAS-2 checklists[23]. Heterogeneity of studies may be explained by the commercial brand of the index test, type of reference standard, disease prevalence, and study size, and risk of bias. Articles that did not contain the indicators of the high risk of bias and inclusion applicability concerns(the degree to which the studies are applicable to the research question) were regarded as a high-quality study[24, 25].

Data Analysis

Data were first extracted into a Microsoft Excel spreadsheet and sensitivity, specificity, positive and negative likelihood ratios, and diagnostic odds ratio were calculated using Meta-DiSc software, developed by *Clinical Biostatistics Unit – Hospital Ramón y Cajal, Hospital University of Madrid, Spain* and results were presented into summary tables and forest plot. Besides, the receiver operating characteristic (ROC) plot was also generated, using sensitivity on the Y-axis and 1-specificity on the X-axis, to classify the patient into VL and non-VL.

Results

Using the search terms, 340 and 322 articles from PUBMED, EMBASE, and Google scholar online databases respectively were retrieved. Besides, 2 more articles were obtained by personal communications and by checking the references of the included articles. After removing duplicates from the three databases with *EndNote X8 reference manager* and initial selection was based on reading the titles and abstracts, 77 articles were further screened by reading the full-text length. Finally, 12 articles were included in the meta-analysis. From the included 12 articles, 2240 diagnostic data were included in the final random-effects models of meta-analysis, (Fig. 2).

The systematic review showed the sensitivity of the rk39-based RDT for VL diagnosis in Ethiopia ranged from 27.8% [9] to 98.3% [21]. Similarly, the specificity was also varied from 27.8% [20] to 98.5% [17], (Table 1*).* The pooled sensitivity and specificity of the included articles were also computed using Meta-DiSc software *V. 1.4*.

The overall pooled sensitivity and specificity of the rk39-based RDTs for VL diagnosis in the Ethiopian studies were 88.0 %(95%CI: 86.0–89.0%) and 84.0 %(95% CI: 82.0–86.0%), respectively, (Fig. 3).

The performance of rk39 in the northern part of Ethiopia was compared with studies done in other parts of the country. The sensitivity and specificity of the rk39 in North Ethiopia, a region with highest VL prevalence, were: 86.0% (95% CI: 84.0–88.0%) and 71.0% (95% CI: 75.0–92.0%), respectively. In the other parts of Ethiopia, the sensitivity and specificity were found 100.0% (95% CI: 72.0–100.0%) and 87.0% (95 % CI: 83.0–90.0%), respectively.

The sensitivity and specificity of the DiaMed commercial test kit were 86.9% (95% CI: 84.3–89.1%) and 82.2 % (95% CI: 79.3–85.0%), respectively. The InBios test kit showed a lower sensitivity and but higher specificity: 80.0 % (95% CI: 77.0–82.8%) and 97.4% (95% CI: 95.0–98.8%), respectively. The sensitivity and specificity of the rk39-based RDT using PCR, microscopy and/or NNN culture, and PCR as reference test were determined. The sensitivity and specificity of rk39-based RDTs using serology as reference test were higher 93.3%(95%CI: 89.1–96.2%) and 91.0% (95%CI: 88.4–93.1%) than microscopy and/or NNN culture (86.1% (95% CI: 83.5–88.5%), and 76.4% (95% CI: 72.4–80.2%) and PCR: 89.8% (95% CI: 86.7–92.3%) and 50.0% (95% CI: 0 to 100%)), respectively, (Table 2).

The likelihood ratio of the rk39 was also determined. The overall positive likelihood ratio (LR) were 5.12 (95% CI: 2.93–8.94%), (Fig. 4).

Simlarly, the negative likelihood ratio was also determined: 0.17 (95% CI: 0.09–0.92%, (Fig. 5)

Performance of rk39 among HIV seropositive individuals:

In this meta-analysis, the sensitivity and specificity of rk39 among the HIV positive and negative participants were determined. In HIV negatives, the sensitivity and specificity of the rk39 were 86.5% (95% CI: 81.3–90.7%) and 97.4 % (95% CI: 94.4–99.0%) respectively. The sensitivity and specificity of rk39 among HIV positive VL patients were 82.0% (95%CI: 75.8–87.1%) and 66.7 % (22.3–95.7%) respectively.

The diagnostic odds ratio (DOR) is an important single indicator that explained the diagnostic capability of a test to correctly differentiate patients from the non-patients. The DOR was: 37.94 (95% CI: 14.28 to 100.81), Fig. 6. However, when the two extreme outliers, *Kiros Y et al*[20] and *Endalamaw G et al*[9], were excluded, the DOR is raised to 49.59 (17.79 to 136.26).

In a meta-analysis of diagnostic test accuracy, the summary receiver operating characteristic (SROC) curve analysis is one of the valuable parameters to classify an individual into disease and without the disease[26]. In this meta-analysis, the SROC curve was 93.4, Fig. 7.

Discussion

In this systematic review, the diagnostic performance of the rk39 varies remarkably, from very low sensitivity (27.8%) [6] to a very high (98.3%) [14]. Similarly, the specificity also showed a noticeable variation from 27.8% [13] to 98.5% [11]. The variation of the rk39-based RDTs might be explained by several factors such as differences in study design, the commercial brand of the rk39-based RDT, types of reference standards, and the presence of other comorbidities like HIV.

The pooled sensitivity of the rk39 based tests assessed in the present meta-analysis was lower (88.0%) compared to the Indian sub-continent (97%) and the global sensitivity (91.9% [27]. In contrast, the result of this meta-analysis was a little higher than the pooled sensitivity of east African studies (85.3%). The variation may possibly be explained by the commercial rk39-based RDTs type differences and the reference test used. More importantly, the sensitivity of the kit in the northern part of Ethiopia, a region with the highest VL burden, is lower (86.0%) compared to the sensitivity in other parts of Ethiopia (100.0%). The possible explanation of these disparities might be species difference, as the predominant circulating species in east African countries is *L. donovani*[28, 29], while the rk39 test is based on the *L. infantum*.

The specificity and specificity of rk39-based RDTs were higher (93.3% and 91.0%) than studies used microscopy and/or NNN culture (86.1% and 76.4%) and polymerase chain reaction (PCR) referrence tests (89.8% and 50.0%). The specificity of rk39-based RDTs to diagnose VL was remarkably lower with wide range of the 95% confident interval (50.0%, 95% CI: 0 to 100%). The possible explanation could be *L. donovani* complex can non-specifically activate B cells to produce cross-reactive antibodies that can affect the specificity of the test[30]. The impact of HIV on the performance of rk39 was also determined and the specificity of rk39 among HIV negative was higher (97.4%) than HIV positive (66.7%) VL patients. However, the sensitivity of HIV positive (86.5%, 95%CI: 83.1–90.7%) and negative VL (82.0%, 95%CI: 75.8 to 87.1%) patients almost remained similar. These disparities could be by the impact of HIV on the depletion of T cells, induction of immune tolerance, and lowering the renewal of the T-cell repertoire which leads to exhaustion of B cells response [31].

Diagnostic odds ratio (DOR), which is not affected by disease prevalence, is an important single quantitative parameter that revealed the tests' ability to classify the individuals into diseased and not diseased [32]. In the present meta-analysis, the DOR of rk39 to diagnose VL was37.9 and therefore the odds of VL patients having a positive rk39 test result is 38 times higher than those individuals without the disease. The likelihood ratio is also another essential indicator for a diagnostic test to assess how likely the VL patients have a positive diagnostic result [33]. Likelihood ratios range from zero to infinity, so the higher the value, the more likely the patient to have the disease. In the present meta-analysis, the positive likelihood ratio was 5.12 and hence the positive test result occurs 5.12 times more frequent in VL patients than the non-VL patients. Similarly, the negative likelihood ratio was 0.17 and hence, rk39 negative test result was 1/0.17 = 5.9 times less frequent in VL patients than the non-VL patients. Moreover, another essential indicator of the performance of a diagnostic test is the summary receivers operating characteristic curve (sROC curve), which categorized patients into VL patients and non-VL, which is expressed by the area under the ROC Curve (AUC). The AUC has different scales; 0.9 to 1.0 = excellent, 0.8 to 0.9 = good, 0.7 to 0.8 = fair, and < 0.5 have no diagnostic value. Therefore, in the present meta-analysis, the AUC is 0.93 and hence, according to the result, rk39 is an excellent alternative diagnostic test for VL in endemic remote areas, Fig. 5.

Conclusion

Though the individual studies showed great variations, the pooled sensitivity and specificity of rk39-based RDTs are very good. Therefore, considering the rapidity, simplicity, not require special equipment and electricity, and affordable prices, it is advisable to continue use as a primary test for VL at least at the very remote endemic areas of health centers of Ethiopia.

Declarations

Ethical Approval and Consent to participate

“Not applicable”

Consent for publication

“Not applicable”

Availability of data and materials

Not Applicable meta

Conflict of interests

All authors declared no conflict of interests.

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Author Contributions:

DGH, DW, HDFHS design, and conceptualization of the study. DGH and DW performed the search, retrieved, and made the initial and secondary selection. Methodological and quality assessment, and meta-analysis was of was done by DGH and DW. Manuscript writing and reviewing were done by DGH, DW, MA, YK, and HDFHS.

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References

1. Ferede G., Diro E., Getie S., Getnet G., Takele Y., Amsalu A., et al. Visceral Leishmaniasis-Malaria Coinfection and Their Associated Factors in Patients Attending Metema Hospital, Northwest Ethiopia: Suggestion for Integrated Vector Management. Malar Res Treat. 2017;2017:6816913.
2. Diro E., Techane Y., Tefera T., Assefa Y., Kebede T., Genetu A., et al. Field evaluation of FD-DAT, rK39 dipstick and KATEX (urine latex agglutination) for diagnosis of visceral leishmaniasis in northwest Ethiopia. Trans R Soc Trop Med Hyg. 2007;101(9):908–914.
3. de Ruiter C. M., van der Veer C., Leeflang M. M., Deborggraeve S., Lucas C., Adams E. R. Molecular tools for diagnosis of visceral leishmaniasis: systematic review and meta-analysis of diagnostic test accuracy. J Clin Microbiol. 2014;52(9):3147–3155.
4. Elmahallawy E. K., Martínez A. S., Rodriguez-Granger J., Hoyos-Mallecot Y., Agil A., Mari J. M. N., et al. Diagnosis of leishmaniasis. J Infect Dev Ctries. 2014;8(8):961–972.
5. ter Horst R., Tefera T., Assefa G., Ebrahim A. Z., Davidson R. N., Ritmeijer K. Field evaluation of rK39 test and direct agglutination test for diagnosis of visceral leishmaniasis in a population with high prevalence of human immunodeficiency virus in Ethiopia. Am J Trop Med Hyg. 2009;80(6):929–934.

6. Alebie G., Worku A., Yohannes S., Urga B., Hailu A., Tadesse D. Epidemiology of visceral leishmaniasis in Shebelle Zone of Somali Region, eastern Ethiopia. Parasit Vectors. 2019;12(1):209.
7. Ayelign B., Jemal M., Negash M., Genetu M., Wondmagegn T., Zeleke A. J., et al. Validation of in-house liquid direct agglutination test antigen: the potential diagnostic test in visceral Leishimaniasis endemic areas of Northwest Ethiopia. BMC microbiology. 2020;20:1–7.
8. Diro E., Lynen L., Assefa M., Takele Y., Mengesha B., Adem E., et al. Impact of the use of a rapid diagnostic test for visceral leishmaniasis on clinical practice in Ethiopia: a retrospective study. PLoS Negl Trop Dis. 2015;9(5):e0003738.
9. Gadisa E., Custodio E., Canavate C., Sordo L., Abebe Z., Nieto J., et al. Usefulness of the rK39-immunochromatographic test, direct agglutination test, and leishmanin skin test for detecting asymptomatic Leishmania infection in children in a new visceral leishmaniasis focus in Amhara State, Ethiopia. Am J Trop Med Hyg. 2012;86(5):792–798.
10. Akhoundi M., Downing T., Votypka J., Kuhls K., Lukes J., Cannet A., et al. Leishmania infections: Molecular targets and diagnosis. Mol Aspects Med. 2017;57:1–29.
11. Srivastava P., Dayama A., Mehrotra S., Sundar S. Diagnosis of visceral leishmaniasis. Trans. R. Soc. Trop. Med. Hyg. 2011;105(1):1–6.
12. Dixit K. K., Verma S., Singh O. P., Singh D., Singh A. P., Gupta R., et al. Validation of SYBR green I based closed tube loop mediated isothermal amplification (LAMP) assay and simplified direct-blood-lysis (DBL)-LAMP assay for diagnosis of visceral leishmaniasis (VL). PLoS Negl Trop Dis. 2018;12(11):e0006922.
13. Maia Z., Lírio M., Mistro S., Mendes C. M. C., Mehta S. R., Badaro R. Comparative study of rK39 Leishmania antigen for serodiagnosis of visceral leishmaniasis: systematic review with meta-analysis. PLoS neglected tropical diseases. 2012;6(1).
14. Boelaert M., Lynen L., Desjeux P., Van Der Stuyft P. Cost-effectiveness of competing diagnostic-therapeutic strategies for visceral leishmaniasis. Bulletin of the World Health Organization. 1999;77(8):667.
15. Burns J. M., Shreffler W. G., Benson D. R., Ghalib H. W., Badaro R., Reed S. G. Molecular characterization of a kinesin-related antigen of Leishmania chagasi that detects specific antibody in African and American visceral leishmaniasis. Proceedings of the National Academy of Sciences. 1993;90(2):775–779.
16. Vaish M., Singh O. P., Chakravarty J., Sundar S. rK39 antigen for the diagnosis of visceral leishmaniasis by using human saliva. The American journal of tropical medicine and hygiene. 2012;86(4):598–600.
17. Canavate C., Herrero M., Nieto J., Cruz I., Chicharro C., Aparicio P., et al. Evaluation of two rK39 dipstick tests, direct agglutination test, and indirect fluorescent antibody test for diagnosis of visceral leishmaniasis in a new epidemic site in highland Ethiopia. Am J Trop Med Hyg. 2011;84(1):102–106.
18. Al Borzi A., Rasouli M., Nademi Z., Kadivar M., Pourabbas B. Evaluation of rK39 strip test for the diagnosis of visceral leishmaniasis in infants. EMHJ-Eastern Mediterranean Health Journal, 12 (3–4), 294–299, 2006. 2006.
19. Ghasemian M., Gharavi M. J., Akhlaghi L., Mohebali M., Meamar A. R., Aryan E., et al. Development and Assessment of Loop-Mediated Isothermal Amplification (LAMP) Assay for the Diagnosis of Human Visceral Leishmaniasis in Iran. Iran J Parasitol. 2014;9(1):50–59.
20. Kiros Y. K., Regassa B. F. The role of rk39 serologic test in the diagnosis of visceral leishmaniasis in a Tertiary Hospital, Northern Ethiopia. BMC Res Notes. 2017;10(1):169.
21. Ejazi S. A., Ghosh S., Saha S., Choudhury S. T., Bhattacharyya A., Chatterjee M., et al. A multicentric evaluation of dipstick test for serodiagnosis of visceral leishmaniasis in India, Nepal, Sri Lanka, Brazil, Ethiopia and Spain.

Sci Rep. 2019;9(1):9932.

22. Bezuneh A., Mukhtar M., Abdoun A., Teferi T., Takele Y., Diro E., et al. Comparison of point-of-care tests for the rapid diagnosis of visceral leishmaniasis in East African patients. Am J Trop Med Hyg. 2014;91(6):1109–1115.
23. Whiting P. F., Rutjes A. W., Westwood M. E., Mallett S., Deeks J. J., Reitsma J. B., et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Annals of internal medicine. 2011;155(8):529–536.
24. Nagai K., Horita N., Yamamoto M., Tsukahara T., Nagakura H., Tashiro K., et al. Diagnostic test accuracy of loop-mediated isothermal amplification assay for Mycobacterium tuberculosis: systematic review and meta-analysis. Scientific reports. 2016;6:39090.
25. Venazzi A., Swardfager W., Lam B., de Oliveira Siqueira J., Herrmann N., Cogo-Moreira H. Validity of the QUADAS-2 in Assessing Risk of Bias in Alzheimer's Disease Diagnostic Accuracy Studies. Front. Psychiatry. 2018;9.
26. Narkhede S. Understanding AUC-ROC Curve. Towards Data Science. 2018;26.
27. Boelaert M., El-Safi S., Hailu A., Mukhtar M., Rijal S., Sundar S., et al. Diagnostic tests for kala-azar: a multi-centre study of the freeze-dried DAT, rK39 strip test and KAtex in East Africa and the Indian subcontinent. Trans R Soc Trop Med Hyg. 2008;102(1):32–40.
28. Al-Salem W., Herricks J. R., Hotez P. J. A review of visceral leishmaniasis during the conflict in South Sudan and the consequences for East African countries. Parasites & vectors. 2016;9(1):460.
29. Leta S., Dao T. H. T., Mesele F., Alemayehu G. Visceral leishmaniasis in Ethiopia: an evolving disease. PLoS Negl Trop Dis. 2014;8(9):e3131.
30. Galvao-Castro B., Ferreira J. S., Marzochi K., Marzochi M., Coutinho S., Lambert P. Polyclonal B cell activation, circulating immune complexes and autoimmunity in human american visceral leishmaniasis. Clinical and experimental immunology. 1984;56(1):58.
31. Lindoso J. A. L., Moreira C. H. V., Cunha M. A., Queiroz I. T. Visceral leishmaniasis and HIV coinfection: current perspectives. Hiv/aids (Auckland, NZ). 2018;10:193.
32. Glas A. S., Lijmer J. G., Prins M. H., Bonsel G. J., Bossuyt P. M. The diagnostic odds ratio: a single indicator of test performance. Journal of clinical epidemiology. 2003;56(11):1129–1135.
33. Geweke J. F., Singleton K. J. Interpreting the likelihood ratio statistic in factor models when sample size is small. Journal of the American Statistical Association. 1980;75(369):133–137.

Tables

Table 1

Compiled Eligible Studies Included in the Meta-Analysis of rk39 in Ethiopia.

Author	Year	TP	FN	FP	TN	Ref. test	Control group	Sens (95% CI)	Spec (95% CI)
Asrat B et al HIV- (DiaMed)	2014	80	2	2	109	DAT	Healthy non-endemic, Healthy endemic & Other disease control	0.976(0.98–0.99)	0.982(0.93–0.99)
Asrat B et al HIV- (InBios)	2014	77	5	2	109	DAT	Healthy non-endemic, Healthy endemic & Other disease control	0.939(0.88–0.97)	0.982(0.98 – 0.93)
Asrat B et al HIV+ (DiaMed)	2014	12	1	0	0	DAT	Healthy non-endemic, Healthy endemic & Other disease control	0.893(0.60–0.98)	0.500(0.02–0.98)
Asrat B et al HIV+ (InBios)	2014	11	2	0	0	DAT	Healthy non-endemic & Healthy endemic & Other disease control	0.821(0.54–0.95)	0.500(0.02–0.98)
Boelaert M, et al (InBios)	2008	15	5	5	10	DAT	Endemic patient control	0.750(0.52–0.89)	0.667(0.41–0.85)
Carmen C, et al (DiaMed)	2011	32	3	4	63	Microscopy	Healthy endemic control	0.914(0.77–0.97)	0.940(0.85–0.98)
Carmen C, et al (InBios)	2011	33	2	1	66	Microscopy	Health endemic control	0.943(0.80–0.99)	0.985(0.90–0.99)
Diro E et al (DiaMed)	2015	165	22	72	104	Microscopy&NNN	Endemic patient control	0.882(0.83–0.92)	0.591(0.52–0.66)
Diro E et al (InBios)	2007	35	14	9	43	Microscopy&NNN	Endemic patient control	0.714(0.57–0.82)	0.827(0.70–0.91)

Author	Year	TP	FN	FP	TN	Ref. test	Control group	Sens (95% CI)	Spec (95% CI)
Kiros et al(InBios)	2017	37	7	13	5	Microscopy	Endemic patient control	0.841(0.70–0.92)	0.278(0.12–0.52)
ter Horst et al, HIV+ (DiaMed)	2009	136	32	2	9	Micoscopy	Other diseased patients	0.810(0.74–0.86)	0.818(0.49–0.95)
ter Horst et al, HIV- (DiaMed)	2009	42	23	2	4	Microscopy	Other diseased patients	0.646(0.52–0.75)	0.667(0.27–0.92)
Sarfaraz et al (InBios)	2019	85	1	0	0	Microscopy	Other diseased patient & healthy endemic	0.983(0.92–0.99)	0.500(0.02–0.98)
Getachew et al (DiaMed)	2019	11	0	46	304	*ELISA	Endemic Patient control	0.958(0.58–0.99)	0.868(0.82–0.90)
Endalamaw et al (InBios)	2012	2	6	0	23	DAT	Endemic patient control	0.278(0.08–0.62)	0.979(0.74–0.99)
Canavate C et al (Diamed)	2011	223	23	0	0	PCR	Healthy endemic	0.905(0.86–0.94)	0.500(0.02–0.98)
Canavate C et al (Inbios)	2011	219	27	0	0	PCR	Healthy endemic	0.889(0.84–0.92)	0.500(0.02–0.98)
Alelign B et al (InBios)	2020	106	4	11	75	Micoscopy	Other diseased patient & healthy control	0.96(0.91–0.99)	0.87(0.78–0.93)

Due to technical limitations, table 2 is only available as a download in the Supplemental Files section.

Figures

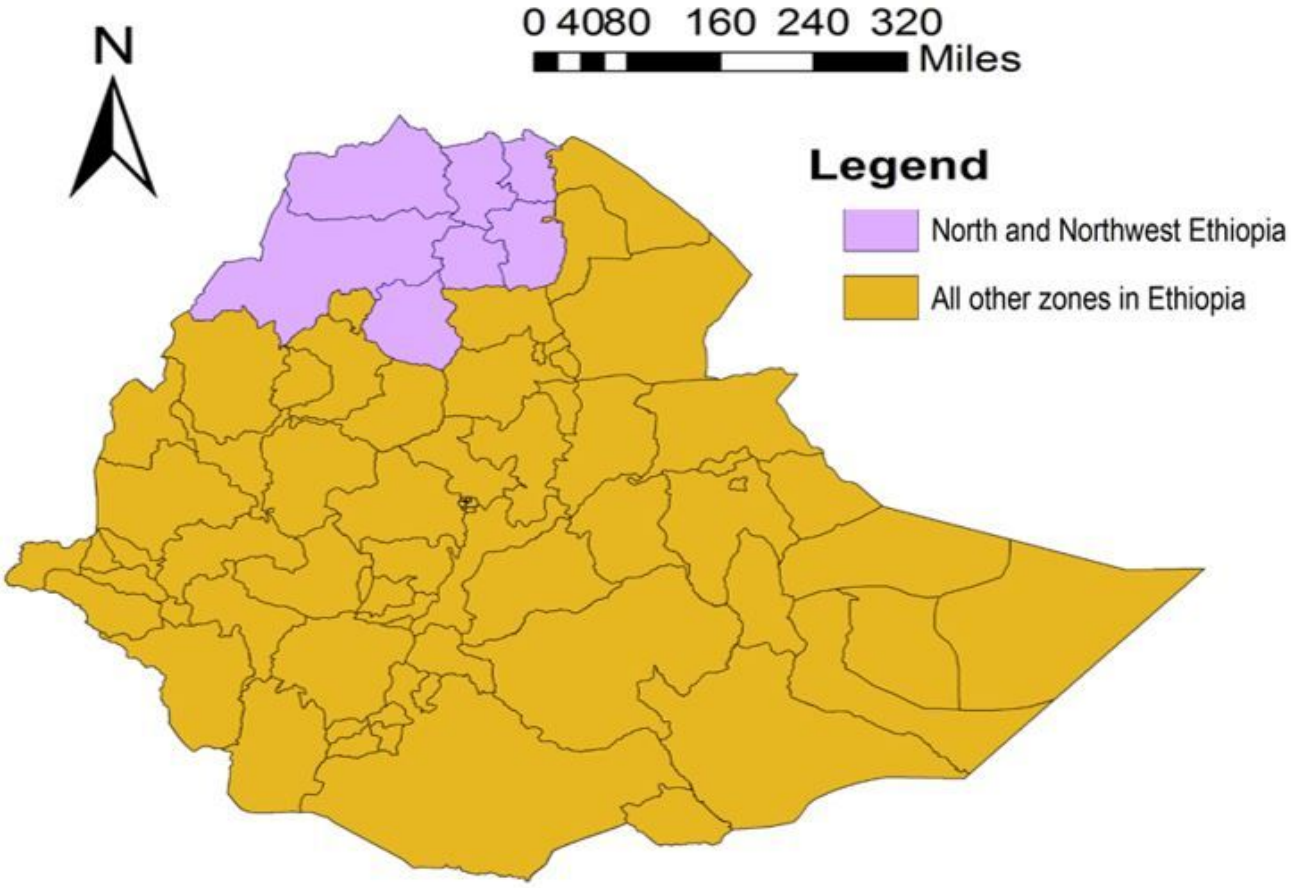

Figure 1

A map showing the north and northwest, and remaing other areas of Ethiopia Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

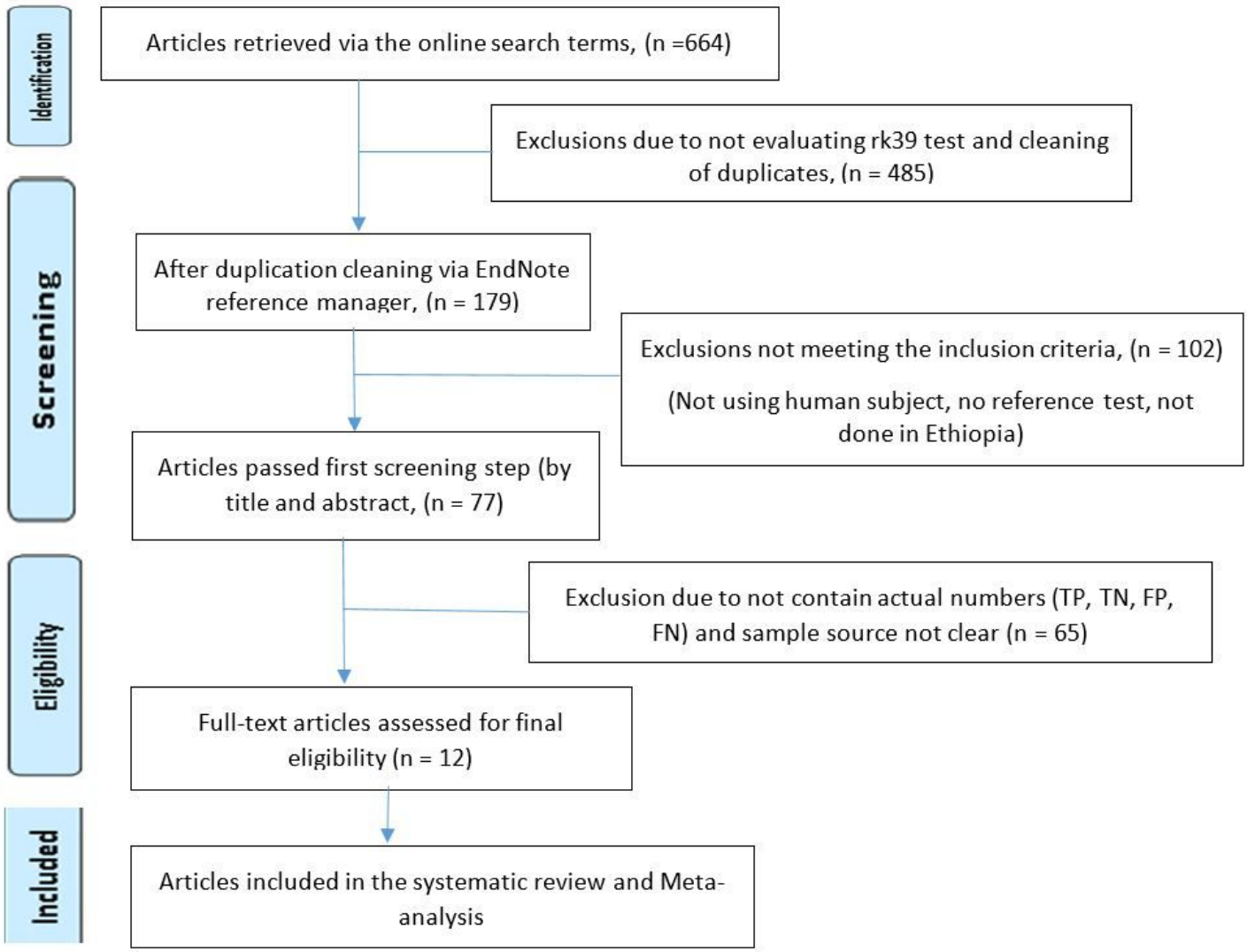

Figure 2

PRISMA Workflow Diagram for the article's selection process

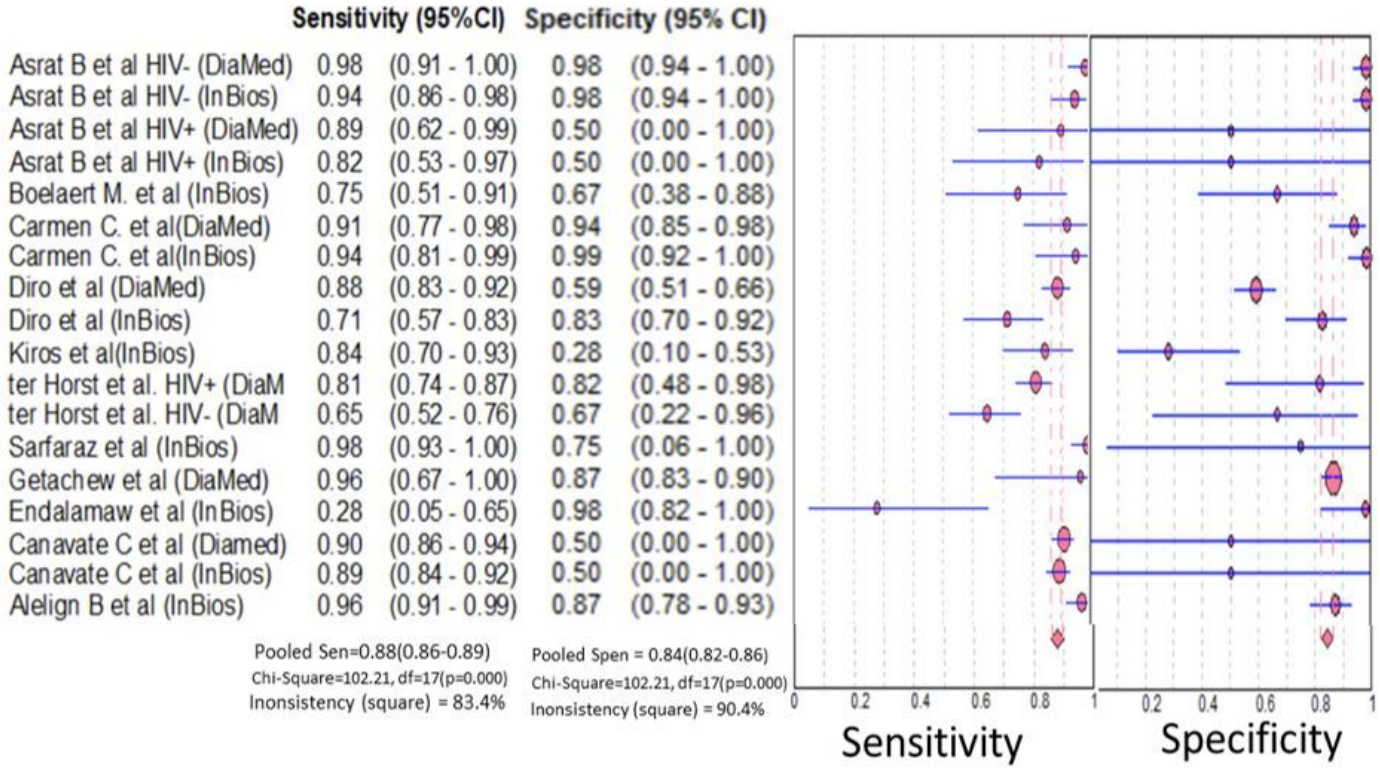

Figure 3

Estimates of sensitivity and specificity of rK39-based RDTs for the serodiagnosis of VL in Ethiopian studies.

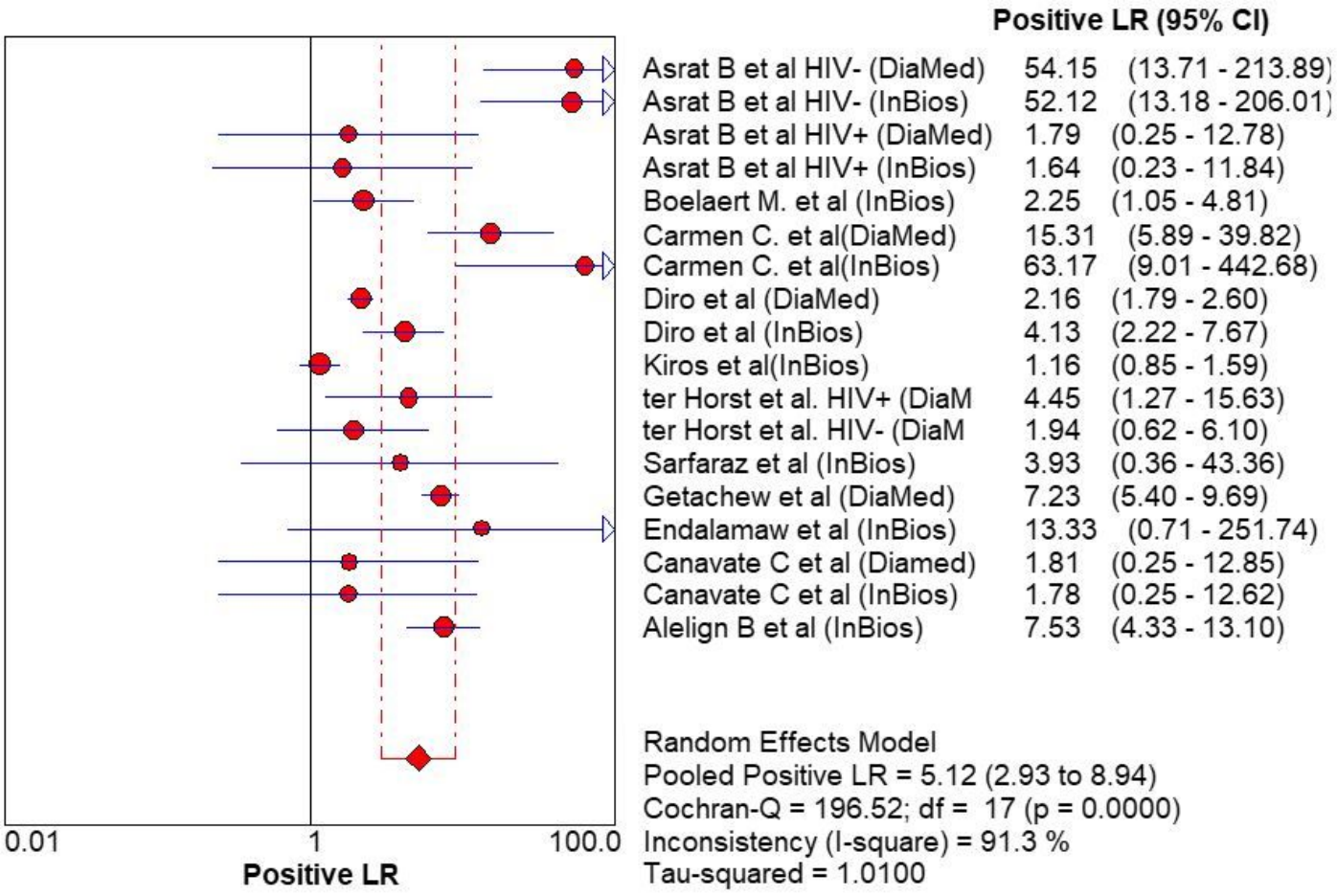

Figure 4

Estimates of positive likelihood ratio of rK39 for the diagnosis of VL in Ethiopian studies

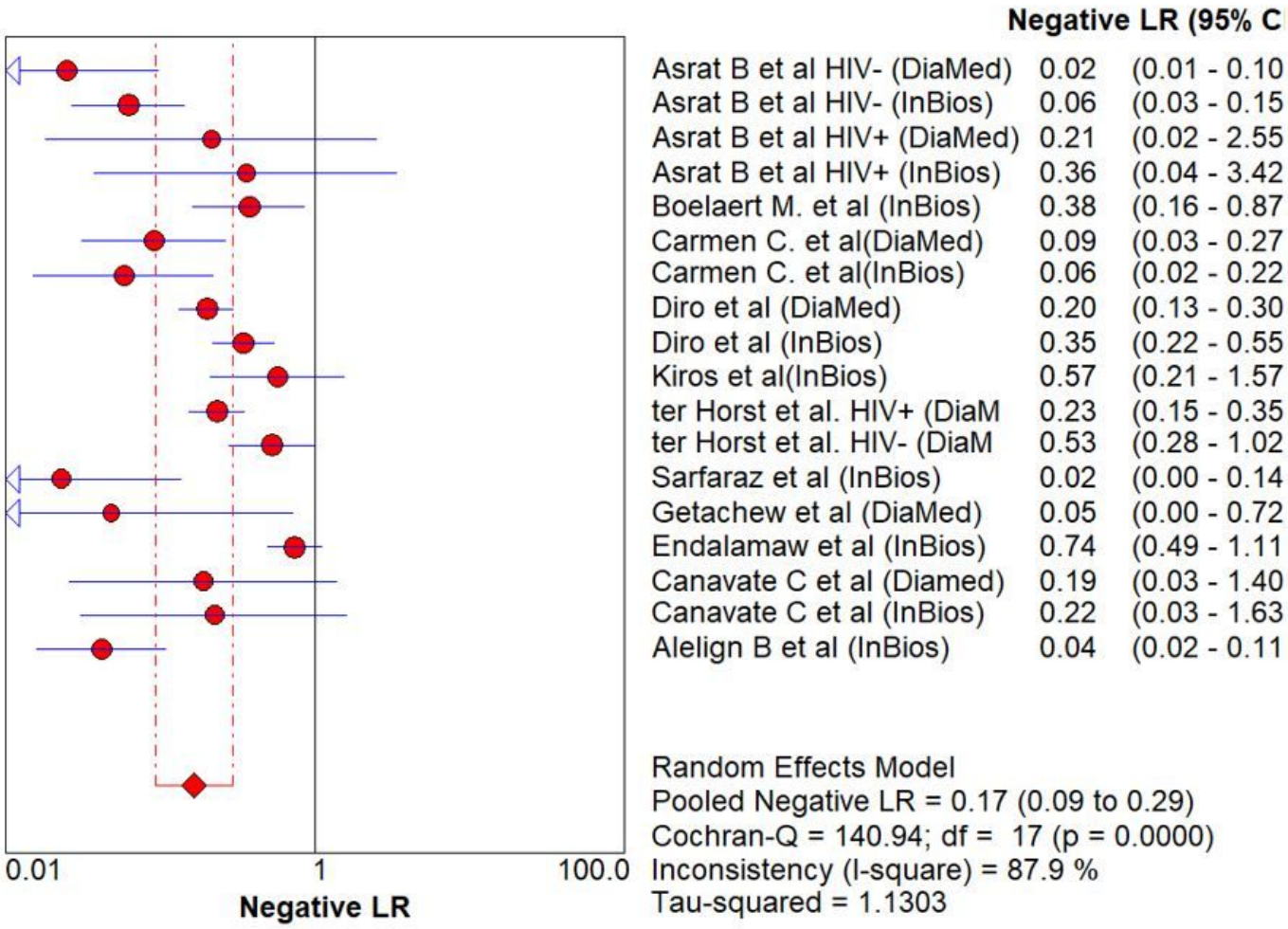

Figure 5

Estimates of negative likelihood ratio of rK39 for the diagnosis of VL in Ethiopian studies

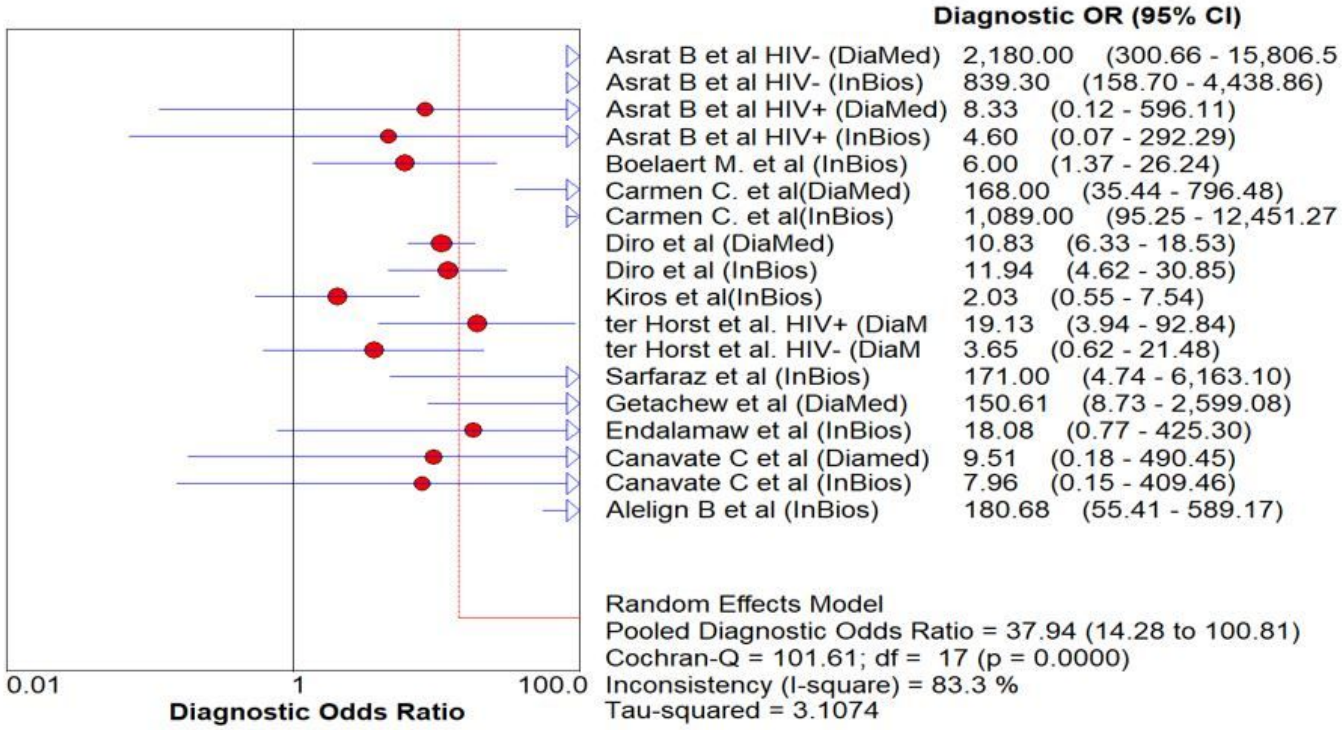

Figure 6

Diagnostic Odds Ratio of rk39 to diagnose VL in Ethiopia

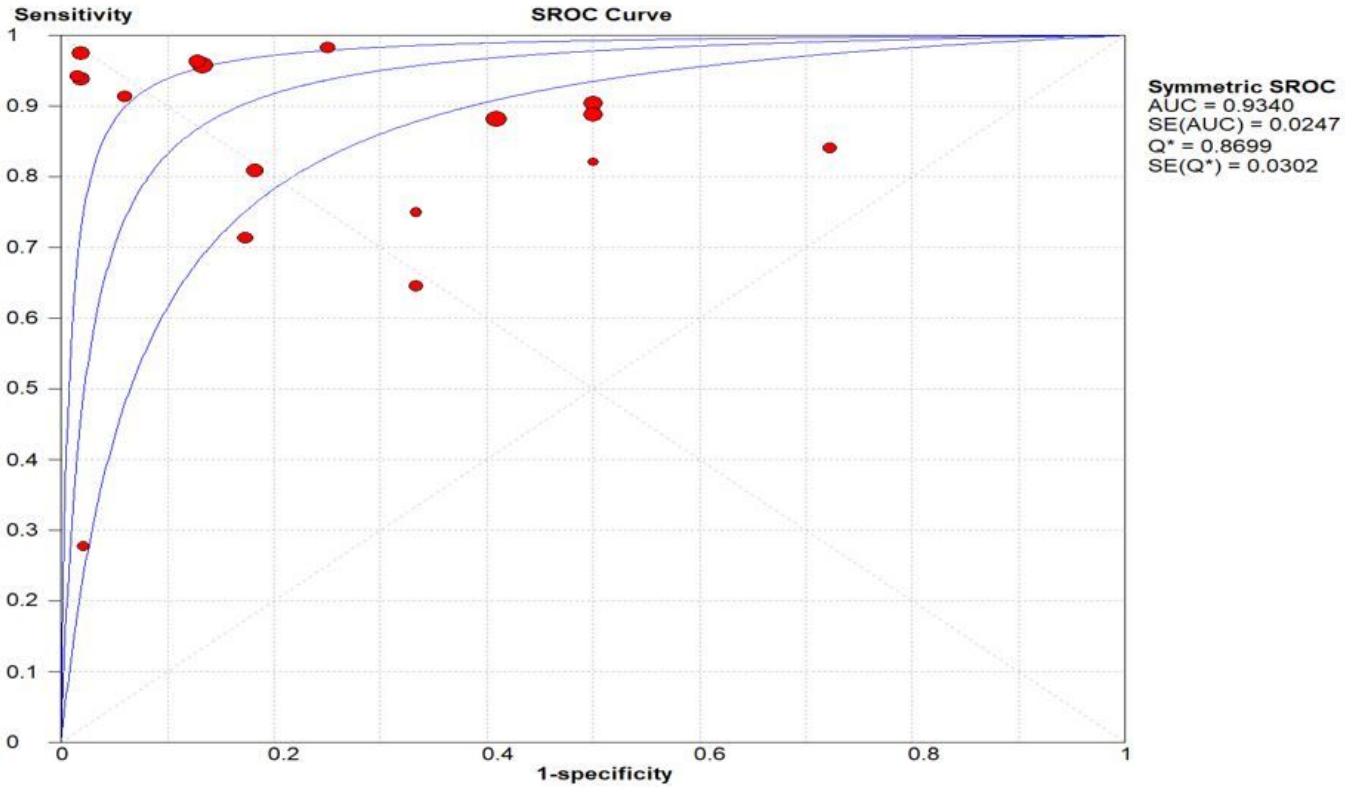

Figure 7

summary ROC of rk39 for the diagnosis of VL in Ethiopia.

Supplementary Files

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- Table2.jpg