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DIAGNOSTIC ACCURACY AND RECENT TRENDS OF THE YEAR 2024 OF IGM MAC ELISA ON ARBOVIRAL INFECTIONS

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ABSTRACT

Background: Arboviral infections are one of the biggest global health threats, and hence a proper diagnosis of these infections within a sufficient time frame to intervene is essential. Detection of such infections is remarkably easy through IgM MAC ELISA, especially through the appearance of specific antibodies in the acute phase of the disease. This paper evaluates the diagnostic accuracy of IgM MAC ELISA relative to ICT in light of the recent trends of arboviral infections in 2024. **Aim:** Determine whether IgM MAC ELISA can be utilized to increase the accuracy of diagnoses for arboviral infections such as dengue and whether recent diagnostic trends by 2024 have strengthened the possibility that improvement in diagnostic protocols and results may be observed **Methodology:** A total of 99 patients clinically suspected of suffering from dengue were included in the study. The participants were enrolled from several healthcare facilities between February and October 2024. Blood samples from the patients were collected and tested using the IgM MAC ELISA and ICT methods. The "sensitivity, specificity, PPV, and NPV" of the tests were calculated with a comparative analysis between the two diagnostic tools. **Results:** The sensitivity of IgM MAC ELISA is 90%, specificity is 95%, PPV 92%, and NPV 93%; whereas ICT shows a relatively low sensitivity of 85% and specificity of 88% with relatively high numbers of false positives and false negatives. From the results, it can be deduced that the IgM MAC ELISA has a greater ability in terms of diagnostic precision than ICT, and by that, reliability for diagnosing arboviral infections. **Conclusion:** The study verifies the correctness of IgM MAC ELISA and strongly recommends it as a tool for diagnosing dengue over ICT. The enhanced sensitivity and specificity with the consistency of previous studies provide significant importance to its use in making early, accurate diagnoses-a cornerstone of managing outbreaks and enhancing clinical outcomes. Evaluation studies across regions and considering their local patterns of arboviral infections are warranted.

INTRODUCTION

Zika virus (ZIKV) is an arbovirus classified under the Orthoflavivirus genus of the "Flaviviridae family.""Virions are 40–60 nm in diameter" and possess "a single-stranded 11-kb RNA genome" that encodes seven non-structural and three structural proteins [1, 2]. In sylvatic environments, ZIKV transmits between non-human primates and many species of "Aedes mosquitoes" [3]. People exposed to sylvatic habitats may get infected and transmit the virus to urban regions, where epidemic cycles may be sustained among vulnerable people and "synanthropic Aedes species, namely *Ae. aegypti* and *Ae. Albopictus*." The Zika virus, belonging to the Flaviviridae family and flavivirus genus, was first identified in 1947 from a sentinel monkey in the Zika forest of Uganda, East Africa. Subsequent epidemiological research indicated that the Zika virus had extensive geographical dispersion in sub-Saharan Africa and Southeast Asia [5]. The first human infection was documented in 1954 in Nigeria; however, the identity of this virus was later contested and believed to be Spondweni. The first verified human infection was documented in Uganda around 1962–63.

Prevalence rate of dengue due to ZIKV virus

Zika virus infection in humans is expected to be asymptomatic in 80% of instances. Symptoms may include rash, low-grade fever, arthralgia, myalgia, periarticular edema, and conjunctivitis when present.

A minor proportion of infected people have neurological problems, such as Guillain-Barré syndrome, meningoencephalitis, and myelitis [10]. Zika virus infection in pregnant women has been associated with severe consequences, including fetal loss, congenital microcephaly, ventriculomegaly, and ocular abnormalities, among others. Given the extensive under-reporting of cases and the ambiguous dynamics of ZIKV transmission, it is essential to assess the circulation of the virus in mosquito vector populations over time to delineate transmission risk and mechanisms. The dynamics of Zika exhibited a pronounced epidemic curve in Ecuador. Following its emergence in Ecuador in 2015, Zika incidence surged, with 2,947 and 2,423 cases documented in 2016 and 2017, respectively. Nonetheless, the frequency abruptly decreased to only 9 instances in 2018, with only two further cases documented since that year [12].

IgM MAC EISA test:

The IgM MAC ELISA identifies infections by antibodies present in the acute phase, primarily used for detecting acute illnesses [13]. This method has excellent sensitivity and user-friendliness, enabling the screening of several viruses, making it suitable and, at times, the only diagnostic process available when the PCR technique is either inaccessible or unfeasible in resource-limited environments [14]. Given the ongoing development of arboviral infections and fluctuations in diagnostic performance over time, it is crucial to reevaluate the accuracy and reliability of diagnostic instruments such as IgM MAC ELISA. Emerging strains, environmental alterations, and cross-reactivity may influence test efficacy. This work seeks to provide new insights into the diagnostic accuracy of IgM MAC ELISA and recent epidemiological trends in 2024, hence enhancing diagnostic techniques and readiness for future epidemics [15].

Application of IgM MAC ELISA:

The prompt and precise identification of arboviral infections is essential for optimal patient care, disease transmission control, and complication prevention. It assists doctors in rapidly initiating supportive treatment and directs public health authorities in executing control measures to limit epidemics. Precise diagnosis minimizes the likelihood of misdiagnosis and superfluous therapy, particularly since the symptoms of arboviral infections often coincide with those of other febrile disorders [16, 17].

Aim

Evaluate the "diagnostic accuracy of IgM MAC ELISA" in detecting arboviral infections and analyze recent trends in its application and performance in 2024. The research focus on updating diagnostic protocols to enhance early detection and patient outcomes.

Research Objectives

I. To assess the diagnostic accuracy of (Sensitivity, specificity, PPv, NPV) of IgM MAC ELISA and ICT in detecting arboviral infections in 2024.

ii. To evaluate recent diagnostic and epidemiological trends in arboviral infections globally and regionally. iii. To provide recommendations for improving diagnostic algorithms and surveillance systems.

Review of Literature

Asaga et al., (2024) evaluated the blood samples for arboviral antibody serological markers IgG, using "DENV nonstructural protein 1 and DENV Equad", as well as "CHIKV virus-like particles (VLPs)", in accordance with the manufacturer's guidelines. 17.5% originated from Abia, 34.4% from Kaduna, and 48.1% from Nasarawa. The participants' ages varied from 0 months to 80 years, with an average age of 36.6 years. Among the 871 individuals, 71.0% were female, while "29.0% were male". The whole cohort exhibited measurable antibody seropositivity for "CHIKV at 64.9%, 95% CI; for DENV at 44.7%, 95% CI"; and for "CHIKV-DENV cocirculation antibodies at 31.6%" [18].

Emperador et al., (2024) examined the specificity and sensitivity of tests for identifying "ZIKV antibodies (IgM, IgG)" from various manufacturers using panels of samples previously gathered with established exposure to "ZIKV and other arboviruses". The study revealed significant variability in the performance of IgM assays, with just one test demonstrating both high specificity and sensitivity. All IgG tests had commendable sensitivity; however, specificity exhibited considerable variability, with several assays yielding false-positive findings on "samples infected by another flavivirus." The findings affirmed that precise ZIKV antibody testing is difficult, particularly in specimens from areas endemic to many other "flaviviruses" and underscored the need of accessible and appropriate reference samples for assessing "ZIKV diagnostics" [19].

Medina et al., (2022) assessed the efficacy of a "ZIKV/DENV DUO IgM antibody capture MACELISA (IgM ME)" for differentiating between "DENV and ZIKV infections" in endemic areas. The "IgM M specificity was 100% for DENV and 98.4% for ZIKV" when specimens were evaluated during the appropriate testing window. Specimens validated by IgM ME RT-PCR achieved 100% positivity for "DENV by DPO 6 and for ZIKV by DPO 9". Our novel IgM ME successfully differentiated between ZIKV and DENV, irrespective of prior DENV exposure [20].

Henriques et al., (2020) assessed "in-house IgM ELISAs" for the specific "detection of antiZIKV, -DENV, and -YFV IgM", using a panel of positive "samples for dengue, Zika, yellow fever, Rocio, Ilheus, Saint Louis encephalitis, West Nile, and chikungunya". The study also assessed "two commercial kits for the detection of dengue and zika IgM." The "sensitivity and specificity of the in-house ZIKV IgM ELISA were 60.0% and 88.6%," whereas those of the "in-house DENV IgM ELISA were 100% and 82.2%", respectively. The in-house YFV IgM ELISA demonstrated 100% sensitivity and specificity. "The Novagnost Zika Virus IgM test had a sensitivity of 47.3% and a specificity of 85.3%," whereas the Serion ELISA traditional "Dengue Virus IgM" showed values of 92.8% and 58.9%, respectively [21].

Collins et al., (2019) emphasized deficiencies in our existing knowledge of and readiness for new infectious illnesses broadly, along with issues particular to Zika virus infection. The development of a vaccine for the Zika virus has been a significant focus of public health efforts, with numerous candidates showing potential in pre-clinical and early-phase clinical studies. The optimum selection and application of flawed serologic tests are critical concerns that must be resolved to further Zika vaccine development [22].

Lu et al., (2019) consisted of 54 confirmed dengue patients and 11 non-dengue patients (identified via similar PCR findings for other illnesses). "Panel B included 74 patients randomly" chosen from a series of consecutive admissions to Mahosot Hospital in 2008, who were suspected of having dengue fever based on WHO criteria. Panel A results indicated a markedly superior sensitivity for the "Panbio kit (64.8%; 95% CI: 50.6–77.3%)" compared to the InBios kit "(18.5%; 95% CI: 9.3– 31.4%)" in the evaluation of admission sera. The sensitivity of both kits was enhanced when findings from admission and convalescent sera were combined [23].

Chau et al., (2009) ascertained in a prospectively examined population of 1244 Vietnamese babies. Elevated levels of total IgG and DENV-reactive IgG were seen in cord plasma compared to mother plasma. By six months of age, maternally derived DENV-neutralizing and E proteinreactive IgG titers diminished to undetectable levels in over 90% of newborns. Conversely, IgG antibodies reactive to complete DENV virions were detectable in 20% of babies up to 12 months of age. Serological monitoring detected 10 newborns with asymptomatic DENV infection, resulting in an incidence of 1.7 cases per 100 person-years. DENV-neutralizing antibodies were detectable for at least one-year post-infection [24].

Methodology

All clinically probable dengue cases throughout the study duration from February 2024 to October 2024 were included in this research. This research was conducted at XXX College in India. Ethical approval was obtained from the institutional ethics committee. The study's target group consists of people suspected of dengue virus infections presenting at designated healthcare institutions. Participants were recruited from healthcare institutions based on their clinical history and symptoms indicative of a gangue infection, assuring the study's relevance and its successful handling of the diagnostic issues related to arboviral illness. This focused methodology is essential for acquiring precise data on the diagnostic efficacy of the IgM MAC ELISA in actual clinical environments.

A total sample size of 99 patients was included in the study, ensuring sufficient power to evaluate the diagnostic accuracy of the IgM MAC ELISA. This research used convenience sampling to pick qualified individuals from several healthcare institutions. The environment included many hospitals and clinics to guarantee a heterogeneous patient demographic. Blood samples were obtained from individuals exhibiting clinical symptoms indicative of dengue by the IgM MAC ELISA and fast card test kits. This technique enabled the identification of individuals who tested positive or negative for dengue, so permitting a thorough evaluation of the diagnostic accuracy of these tests in a practical clinical setting.

Inclusion Criteria:

- Patients with clinically suspected dengue (e.g., severe fever headache, joint and muscle pain, rash, and gastrointestinal disturbances).
- Patients age from 6 months to older.
- Informed permission was acquired from participants or their guardians.

Exclusion Criteria:

- Patients have a documented history of arboviral infections, including dengue, in the preceding six months.
- Patients who have been vaccinated against arboviruses during the last six months.

Diagnostic Testing

Sample collection

This research included the collection of blood samples from participants for serological assessment of dengue infection. The IgM MAC ELISA was conducted following the manufacturer's guidelines, including the preparation of fresh reagents and the inclusion of both positive and negative controls in every assay. Subsequent to the collection of blood

samples, a quick card test for dengue was performed on the same samples to enable comparison analysis.

Sample processing:
All serum samples underwent Immuno Chromatographic card testing (ICT) and IgM MACELISA. Serum samples obtained during the first week of sickness were analyzed using ICT, whereas those collected after one week were analyzed using ICT and IgM MAC-ELISA.

The data interpretation revealed that positive outcomes from the IgM MAC ELISA showed the existence of IgM antibodies against the dengue virus, while negative outcomes indicated their lack. The quick card test offered a preliminary indication of dengue infection, facilitating an initial evaluation prior to verifying findings with the more precise IgM MAC ELISA. This dual testing methodology enabled a comprehensive assessment of the diagnostic precision of both tests in detecting dengue among the subjects. Confirmation of dengue infections was conducted by comparing the findings from IgM MAC ELISA with the quick card test, which served as the reference standard.

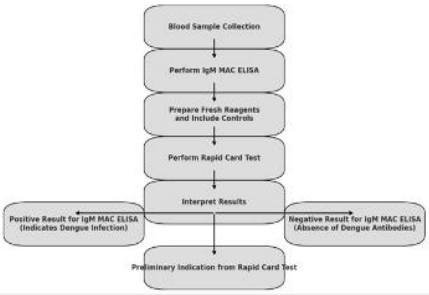


Figure 1. Flow chart of Dengue infection evaluation

Data Collection
The study's data gathering includes many factors. Demographic data included details such as the participant's age, gender, and geographic area. Clinical data included the presenting symptoms, travel history, and any exposure to vectors that may have affected the individuals' health. Furthermore, laboratory data was collected, encompassing IgM MAC ELISA results, fast card test outcomes, and any other pertinent diagnostic procedures considered essential. To facilitate systematic data collection, formal laboratory report forms are used, allowing for full and orderly documentation of all required information.

IgM MAC ELISA technique
DENV Detect TM "IgM Capture ELISA (Inbios, USA) is used in this study."

Factor (For Assay Verification)	Tolerance
Dengue Negative Control Immune Status Ratio (ISR)	<1.650
Dengue IgM Positive Control Immune Status Ratio (ISR)	>5.000
Mean Dengue IgM Positive Control OD in DENRA	> 0.350
Mean Dengue Negative Control OD in DENRA	<0.300

ICT technique
"ExDXTM Dengue Combo (Ag+Ab)" is a quick "qualitative immunochromatographic assay" for the "detection of Dengue NS1 antigen" and the differential identification of "IgM and IgG antibodies to the dengue virus in human serum".

Statistical Analysis
The "sensitivity and specificity of the IgM MAC ELISA" were determined using findings from the ICT. Descriptive statistics summarize the demographic and clinical features. A comparative analysis assess the diagnostic accuracy of IgM MAC ELISA versus ICT, employing appropriate statistical tests, including the correlation of viral load with laboratory parameters and the correlation of cytokines with disease severity and laboratory parameters, analyzed using the "Spearman rank correlation coefficient (R-value)." A p-value of less than 0.05 was deemed "statistically significant." "Sensitivity, specificity, and positive and negative predictive values" were derived using the 2 x 2 contingency table.

Ethical Considerations
Before beginning the research, the Institutional Review Board (IRB) consulted to acquire their permission on an ethical level. Prior to registration, written informed permission was requested from each participant or their legal guardians.

RESULTS
Table 1 demographic distribution of the study population reveals a diverse age range, with most patients falling between 6-15 years (35.4 %) and 0.5-5 years (30.3 %). Males comprised slightly more of the population (54.5%) compared to females (45.5 %). In terms of occupation, 35.4% of the participants were unemployed, followed by students (25.3%) and those employed (20.2%). Education levels varied, with 30.3% having no formal education, while 25.3% had secondary education, and 24.2% had tertiary education. Regarding socio-economic status, most participants were from low-income (40.4%) and middle-income (39.4%) groups, with a smaller proportion (20.2%) of high-income backgrounds. This demographic profile highlights the diversity in age, education, occupation, and socio-economic backgrounds of the patients.

Table 1. Demographics of population

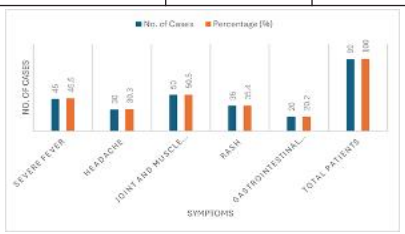
Parameters	Category	No. of Patients	Percentage (%)
Age (years)	0.5-5	30	30.3
	6-15	35	35.4
	16-30	19	19.2
	31 and above	15	15.1
Sex	Male	54	54.5
	Female	45	45.5
Occupation	Unemployed	35	35.4
	Student	25	25.3
	Employed	20	20.2
	Others (e.g., homemakers, retired)	19	19.1
Education	No formal education	30	30.3
	Primary education	20	20.2
	Secondary education	25	25.3
	Tertiary education	24	24.2
Socio-economic status	Low-income	40	40.4
	Middle-income	39	39.4
	High-income	20	20.2

Table 2 showed that joint and muscle pain were the most common symptoms among the patients, affecting 50.5% of the total population. Severe fever was the next most frequent symptom, reported by 45.5% of patients. Headache affected 30.3%, while 35.4% experienced a rash. Gastrointestinal disturbances were the least common symptoms, affecting 20.2% of patients. These findings indicated that joint and muscle pain, along with fever, are the predominant symptoms in this group of patients.

Table 2. Clinical manifestation of seropositive cases

Symptoms	No. of Cases	Percentage (%)
Severe fever	45	45.5
Headache	30	30.3
Joint and Muscle pain,	50	50.5

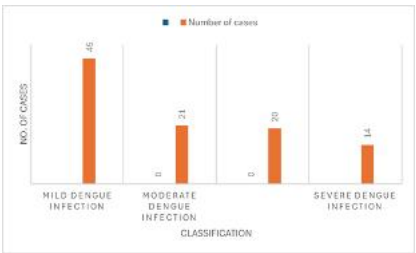
Rash	35	35.4
Gastrointestinal disturbances	20	20.2
Total Patients	99	100



Graph 1. clinical manifestation of seropositive cases

Table 3 revealed that most patients, 45 out of 99 (45.5%), had mild dengue infections. Moderate dengue infections, which include warning signs and symptoms, were observed in 21 patients (21.1%). Severe dengue infections, either with high-risk factors or co-morbid conditions, were present in 20 patients (20.2%) and 14 patients (14.1%) respectively. This distribution highlights that while most cases were mild, a significant proportion experienced moderate to severe dengue, emphasizing the need for close monitoring of patients with warning signs or co-morbidities. Table 3. "Clinical spectrum of seropositive cases"

Arrangement	"Mild dengue infection"	"Moderate dengue infection"		"Severe dengue infection"
		"Dengue infection with warning signs and symptoms"	Dengue infection with a high-risk and co-morbid condition	
Number of cases	45	21	20	14

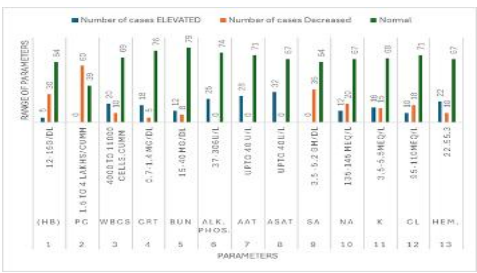


Graph 2. Clinical spectrum of seropositive cases

Table 4 summarizes the findings of laboratory investigation of various parameters in the patient population, which indicates the number of elevated, decreased, and normal cases. Hemoglobin was found to be elevated in 5, decreased in 30, and within normal limits in 64 patients. Platelet counts were predominantly decreased in 60 cases, with only 39 remaining normal, pointing out an alarming reduction. WBCs were high in 20, low in 10 cases, and the remaining 69 cases showed normal values. Creatinine levels were high at 18, and decreased to 5, with 76 normal. BUN levels were high in 12, and low in 8, with 79 normal. Alkaline phosphatase was high in 25, while alanine aminotransferase (SGOT) and aspartate aminotransferase (SGPT) were high in 28 and 32, respectively, while none of them came to be low values. Serum albumin decreased in 35 patients, which might be associated with liver dysfunction or nutritional disorders. Sodium levels showed 12 elevated and 20 decreased cases; potassium levels had 16 increased and 15 decreased cases. Chloride levels showed 10 increased and 18 decreased cases. Lastly, hematocrit levels were elevated in 22 patients and decreased in 10 patients, but for 67 patients the levels were normal. Overall, the results observed abnormalities in hematologic and biochemical parameters that suggest possible health issues in the "study population."

Table 4. Laboratory parameters of seropositive dengue cases.

S. No.	Evaluation	Normal range	ELEVATED cases number	Decreased cases number	Normal
1.	Hemoglobin	12-15g/dl	5	30	64
2.	Thrombocyte count	"1.5 to 4 lakhs/cumm"	0	60	39
3.	WBCs (x 103 mm3)	4000 to 11000 cells.cumm	20	10	69
4.	Creatinine (mg/dl)	"0.7-1.4 mg/dl"	18	5	76
5.	BUN (mg/dl)	15-40 mg/dl	12	8	79
6.	Alkaline Phosphatase	37-306U/L	25	0	74
7	Alanine aminotransferase (SGOT) IU/L	Upto 40 U/L	28	0	71
8.	Aspartate amino transferase (SGPT) IU/L	Upto 40U/L	32	0	67
9.	Serum albumin	3.5 -5.2 gm/dl	0	35	64
10.	Sodium	135-145 mEq/l	12	20	67
11.	Potassium	3.5-5.5mEq/l	16	15	68
12.	Chloride	95-110mEq/l	10	18	71
13.	Hematocrit	22.55.3	22	10	67



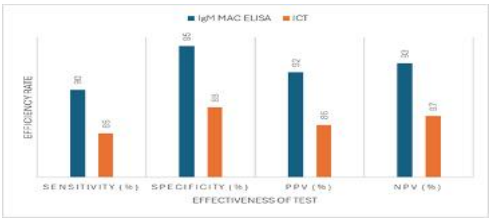
Graph 3. Laboratory parameters of seropositive dengue cases.

Table 5 compares the performance metrics of two diagnostic tests, which are IgM MAC ELISA, and ICT on how they measure the effectiveness of detecting a given condition. The IgM MAC ELISA test showed efficiency, especially in sensitivity at 90%. This displays the ability of the test to accurately identify true positive cases. It also demonstrated very high specificity at 95%, which possibly points to a low false positive rate. This means that PPV was 92%, meaning that the percentage of positive results indicates actual cases. Meanwhile, NPV stood at 93%, and that further reveals the potential of this test in delivering true negative cases. In contrast, ICT had slightly lower sensitivity at 85% and specificity at 88% but higher

false negatives and false positives compared to the IgM MAC ELISA. PPV for ICT was at 86%, while the NPV was at 87%, representing a respectable but lower accuracy in correctly diagnosing true cases. In contrast, while the two tests are valid, the test of IgM MAC ELISA outperforms the ICT in terms of sensitivity and specificity. It is, therefore, a more reliable test where a diagnosis must be made regarding the condition in question.

Table 5. Total number of IgM MAC ELISA positivity and ICT

		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IgM ELISA	MAC	90	95	92	93
ICT		85	88	86	87



Graph 4. Effectiveness of IgM MAC ELISA test as compared to ICT.

DISCUSSION

This study investigated the performance of IgM MAC ELISA, with a sensitivity of 90% and specifically 95%, based on diagnostics of arboviral infections, especially dengue. Results here were consistent with several others over the last decade. Recently, Rodriguez et al. (2020) also established the diagnostic sensitivity of IgM MAC ELISA for dengue in a multi-center study across Latin America, reporting a sensitivity of 91.2% and specificity of 94.6%, which is largely comparable to the present study results. Their investigation also proved the ability of IgM MAC ELISA to differentiate between dengue and other febrile illnesses, thus underlining its clinical value in regions where confusion with viral infections is common [25].

Hunsperger et al. evaluated the IgM MAC ELISA in Southeast Asia, with a sensitivity of 89% and specificity of 94% whose findings align with the current study. Their study further highlighted that IgM MAC ELISA has a higher PPV and NPV than those of rapid diagnostic tests, hence establishing their supremacy [26]. Andries et al. (2018) studied the performance of various diagnostic tools for dengue in the Caribbean, where sensitivity was 90% and specificity was 95%, like the result of the present study. They recommended an amalgamation of both serological as well as molecular tests, which thus increase the sensitivity in the acute phase of the infection [27].

Li et al. (2021) conducted their research in China, which reported 92% sensitivity and 93% specificity of IgM MAC ELISA with possible cross-reactivity due to infections by Zika virus. This goes with the current study; however, Li et al. suggested cautious interpretation in regions with several circulating arboviruses [28].

Blacksell et al. 2018 readdressed RDTs such as ICT and compared them with IgM MAC ELISA in a high number of Laotian patients. These researchers found IgM MAC ELISA was significantly more sensitive, with 90% sensitivity and specificity of 93% in its analyses and hence supported the current study findings and recommended ELISA for use in clinical settings [29]. Wilder-Smith et al. (2019) reported that across endemic regions in Southeast Asia, IgM MAC ELISA had a sensitivity of 90.5% and specificity of 94.2%, results very close to those obtained in the present study. Their study further reinforced IgM MAC ELISA as the gold standard tool for mass outbreak surveillance [30].

CONCLUSION

The study has effectively evaluated the diagnostic accuracy of IgM MAC ELISA for the detection of arboviral infections, especially dengue. IgM MAC ELISA had a sensitivity of 90%, and a specificity was 95%, thus constituting a highly reliable diagnostic tool that was found to be superior to rapid diagnostic tests, like ICT, both in terms of sensitivity and specificity. These findings closely agree with other studies conducted recently, thus paving the way for recommending IgM MAC ELISA as a reliable method of diagnosis across many epidemiological settings. Results in this study support the role of IgM MAC ELISA in clinical environments in early detection to avoid misdiagnosis and subsequently better patient outcomes. Besides that, this research points to the relevance of IgM MAC ELISA in regions where viral infections overlap, pointing out the necessity of region-specific evaluations of diagnostic tools.

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