Original Research Article

A comparison of rapid immunochromatography based tests with ELISA in dengue and leptospirosis in a scenario of 'no rapids'

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Abstract

Background: The blood specimens of patients with fever are tested for Dengue NS1 Antigen (Ag), Dengue IgM/IgG Antibody (Ab) and Leptospira IgM Ab. The specimens are processed by ELISA in routine laboratory and by Rapid Immunochromatographic method (ICT) in Emergency laboratory. **Aims and Objective**: Primary objective is to compare the results of Rapid ICT with the ELISA based tests for the sero-diagnosis of Dengue and Leptospirosis. **Materials and Methods:** A Retrospective study was carried out over a period of 12 months (December 2018 – November 2019). For analysis only results of those samples were compared which were tested by both ICT and ELISA. During the study period, 3802 samples of Dengue NS1, 1384 for Dengue IgM Ab and 2004 for Leptospira IgM Ab satisfied the criteria. **Results:** Considering ELISA as the gold standard, the sensitivity, specificity, PPV and NPV of Dengue NS1 rapid , Dengue IgM antibody rapid and Leptospira IgM antibody rapid were 94.1%, 93.2%, 92.9%, 94.28%; 56.3%, 97.7%, 81.3%, 92.7%; 57.2%,98.5%, 91.2%,91.7% respectively. The discordance rate between rapid and ELISA was 6.4% for Dengue NS1, 8.5% for Dengue IgM and 8.3% for Leptospira IgM. **Conclusion:** Considering the low sensitivity of rapid ICT based tests for antibodies and discordance rate between the rapids and ELISA for NS1 Ag and both IgM Ab kits, the diagnosis of Dengue and Leptospirosis by ELISA should be considered for seamless implementation. Where resources are limited, rapids can still be used until facilities and technical manpower for ELISA testing is available.
Key Words: Dengue, Leptospirosis, Rapid ICT, ELISA, Dengue NSI antigen, IgM antibodies.

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INTRODUCTION

As a part of monsoon protocol, patients presenting with fever, especially during the monsoon and post monsoon period, have their blood specimens tested for various investigations. Of these, specimens are received for Dengue NS1 Antigen (Ag), Dengue IgM/IgG Antibody (Ab) and Leptospira IgM Ab tests. The specimens received during routine hours (9.00am to 4.00pm) are processed by ELISA. After routine hours, till the next morning, the specimens are processed by rapid Immunochromatography (ICT) method. The interim reports of rapid tests are issued within two hours of accepting the specimens. As per the directives of Ministry of Health and Family Welfare, Government of India[1], ELISA is the recommended method of Ag/Ab detection for the diagnosis of Dengue and Leptospirosis. However, ELISA based testing requires need of specialized instruments, skilled manpower, long turnaround time, quality control and batch testing. ICT based tests are performed in the emergency laboratory where there is need for immediate release of reports. The reports are however released as interim reports and the positives are retested by ELISA the next day. It was

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observed that there was discrepancy in the results of the rapid and ELISA tests. This discrepancy in results lead us to formulate a study with the primary objective of comparing the results of Rapid Immunochromatography based tests with the ELISA based tests for the diagnosis of Dengue and Leptospirosis and determining the challenges in implementing an ELISA based testing protocol.

MATERIALS AND METHODS

Ethical Statement

Institutional Ethics Committee approval was obtained (IEC(II)/OUT/93/2020) dated 22nd January 2020.

A retrospective study was carried out for a period of 12 months from December 2018 – November 2019 in the Department of Microbiology at a tertiary care teaching hospital in Mumbai.All specimens received for Dengue and Leptospira serodiagnosis during thisperiod were considered. For analysis only results of those samples were compared which were tested by both ICT and ELISA. During the study period, 3802 samples of Dengue NS1, 1384 for Dengue IgM Ab and 2004 for Leptospira IgM Ab satisfied the criteria.

Study procedure

During routine hours (9.00 am to 4.00 pm), the sero-diagnosis of dengue (NS1/Ab) and leptospirosis (IgM) is based on ELISA. [2,3] The results of ELISA based tests are available the next day. The turnaround time is 24 hours, which increases to 48 hours in case of a holiday. In the emergency laboratory, the diagnosis is based on Rapid Immunochromatography based tests. [2,3] Specimens are received in Emergency laboratory from 4.00 pm in the evening till next day morning 8.00 am. Interim reports are issued within two hours. The positive specimens are retested next working day in the routine laboratory by ELISA method. The results of ICT and ELISA were compared with reference to storage conditions, sample quantity, technical skills, turnaround time (TAT) and cost per test. A confirmed positive report is issued only after the results of the ELISA tests are positive. This study also analysed operational characteristics of both the test formats.

Kits used

1. Dengue NS1 Ag Rapid – Aspen Laboratories Pvt Ltd.,Delhi,India (25 tests/kit)
2. Dengue IgG/IgM Rapid – SD Bioline Dengue IgG/IgM WB by Standards Diagnostics Inc., Republic of Korea (25 tests/kit)
3. Leptospira IgM Rapid – Leptocheck WB by Zephyr biomedicals, Division of Tulip diagnostics Pvt Ltd., India(10 tests/kit)
4. Dengue NS1 Ag ELISA – Recombilisa by CTK biotech Inc., California, USA
5. Dengue IgM ELISA – Trustwell by Athenese diagnostics Pvt Ltd., Chennai, India and CTK biotech, USA
6. Leptospira IgM ELISA – Panbio Leptospira IgM ELISA by Standard Diagnostics Inc. Republic of Korea

The information on the cost of rapid ICT and ELISA kits were collected from central stores and cost per test were calculated.

Quality Assessment:

Quality Control - The quality of both ELISA and rapid kits are verified with known samples prior to being rolled out for testing.For the rapid tests, there is inbuilt control available. However, as per the policy of our department, external known positive and negative controls were tested weekly and with every new lot/batch of kit. The controls for ELISA testing were performed as per the respective kit literature along with one external positive and negative control with each run.

Quality Assurance - The IAMM Dengue serology is undertaken thrice annually. Both Rapid and ELISA tests are performed. Inter-laboratory comparison is also undertaken twice a year for both the tests. The results for the year 2019 were 100% concordant.

Statistical Analysis:

The sensitivity, specificity and predictive validity of rapid test was compared with that of ELISA tests, considering ELISA as reference standard using the standardformulae.

Sensitivity: True positives/(True positives + False negatives) × 100

Specificity: True negatives /(True negatives + False positives) × 100

Positive Predictive Value: **True positives/(True positives + False positives) × 100**

Negative Predictive Value: True negatives / (True negatives + False negatives) × 100

% Discordance of both tests: **(False positives + False negatives) / Total tested × 100**

RESULTS

The administration spent Rs 49/- per ICT based Dengue NS1 Ag test. For Dengue NS1 Ag ELISA it spent Rs 99/- (rainy season) to Rs 105/- (non-rainy season). Rs 75/- was spent on each ICT based Dengue IgM Ab test and Rs 84.5/- (rainy season) to Rs 89.5/- (non-rainy season) was spent on Dengue IgM Ab ELISA test. Rs 124/- was spent on each ICT based Leptospira IgM Ab test, whereas the cost of Leptospira IgM Ab ELISA test varied from 129.5/- (rainy season) to Rs 137/- (non-rainy season)

Table 1: Comparison of Rapid and ELISA tests for Dengue NS1 Ag (n=3802)

		ELISA test		
		Positive	**Negative**	**Total**
Rapid test	Positive	1746	132	1878
	Negative	110	1814	1924
	Total	**1856**	**1946**	**3802**

Sensitivity=94.07%, Specificity=93.22%, PPV=92.97%, NPV=94.28%
% Discordance of both tests= 6.36%

Table 2: Comparison of Rapid and ELISA tests for Dengue IgM Ab (n=1384)

		ELISA test		
		Positive	**Negative**	**Total**
Rapid test	Positive	117	27	144
	Negative	91	1149	1240
	Total	**208**	**1176**	**1384**

Sensitivity=56.25%, Specificity=97.70%, PPV=81.25%, NPV=92.662%
% Discordance of both tests= 8.53%

Table 3: Comparison of Rapid and ELISA tests for Leptospira IgM Ab (n= 2004)

		ELISA test		
		Positive	**Negative**	**Total**
Rapid test	Positive	198	19	217
	Negative	148	1639	1787
	Total	**346**	**1658**	**2004**

Sensitivity=57.22%, Specificity=98.85%, PPV=91.24%, NPV=91.72%
% Discordance of both tests= 8.33%

DISCUSSION

Leptospirosis mainly and Dengue have a seasonal distribution in the city of Mumbai, with maximal number of cases appearing between July to October. The directive by the Government of India, Ministry of Health and Family Welfare (No 7-165/2016/NVBDCP/DEN dated 9th June 2016) mentions use of ELISA test for Dengue NS1antigen and IgM antibody on single serum specimen to confirm the clinically suspected dengue case. A positive result by rapid diagnostic test on a clinically suspected case is to be considered only as probable case[1]. This had led us to think of how to best implement the directives of the Government in a tertiary care medical teaching institute like ours, where there is high number of patient load with limited resources. The ELISA kits required storage at 2-8ºC whereas the rapid kits can be stored at room temperature. For a laboratory with high throughput especially during monsoon, a large capacity low temperature storage device is mandatory coupled with staggered supply. The quality of both ELISA and rapid kits are verified with known samples prior to being rolled out for testing. Availability of trained and competent technicians are required to perform the ELISA tests in the routine laboratory. The resident doctors after undergoing compulsory training and demonstrating competency are allotted duties in emergency laboratory to perform rapid Dengue and Leptospira tests. Availability of trained supervisory staff is required to oversee the ELISA testing and reporting of results. Availability of working ELISA washer and reader and other consumables are necessary for ELISA testing, whereas the consumables required for rapid tests are provided in each kit. Atleast 2- 3ml blood in plain test tube or evacuated tube is required for rapid/ELISA testing. The processing time for ELISA is 3 hours whereas for ICT it is 20 minutes. Also ELISA test needs to be processed in batches, whereas rapid tests are performed on a single specimen. The ELISA test procedure is time-consuming and technically demanding, whereas the rapid tests are easy to perform and less labour intensive. The turnaround time for ELISA test repot is twenty four hours whereas for rapid tests it is two hours. Information regarding cost of the kits was gathered from central stores. The cost per test was calculated. For ELISA tests during the rainy season, one ELISA plate was utilized per day, whereas during non-rainy season, one kit was utilized over two working days. During rainy season eighty nine patient specimens could be tested in one ELISA plate along with five internal controls and two external controls. During non-rainy season, since one kit was utilized over two working days, maximum eighty two patient specimens could be tested per ELISA plate along with the internal and external controls. In the present study, for Dengue NS1 Ag, considering ELISA as gold standard, the rapid ICT test showed a high sensitivity and moderate specificity. The discordance between the two tests was 6.36%. The rapid ICT test for Dengue IgM and Leptospira IgM showed low sensitivity and high specificity. The discordance between the two tests is 8.3% and 8.5% respectively. Considering ELISA as the gold standard, the sensitivity, specificity, PPV and NPV of Dengue NS1 rapid , Dengue antibody rapid and Leptospira IgM rapid were 94.1%,93.2%, 92.9%,94.28%; 56.3%, 97.7%,81.3%, 92.7%; 57.2%,98.5%, 91.2%,91.7% respectively. Similar results have been reported in literature (Table 4) with the sensitivity ranging from 22.5% to 100% and specificity ranging from 50% to 100%.The variable sensitivity and specificity may be the reason why the Government of India circular emphasizes on ELISA as a confirmatory test. In the present study, the Dengue NS1 rapids had higher sensitivity compared to both the IgM rapids.

Table 4: Comparative table of sensitivity and specificity of various rapid ICT kits with ELISA as gold standard in different studies

Author	Journal	Year	Sero target and Kit	Sensitivity (%)	Specificity (%)
Ruta Kulkarni *et al..*[4]	IJMR	2020	NS1, JM RDT	87.3	93.4
			IgM, JMRDT	22.5	93.6
			NS1, SD RDT	93.1	97.8
			IgM, SD RDT	34.4	94.5
Atul Garg and collegues[5]	J Lab Physicians	2019	NS1, Bioline Dengue Duo	100	100
			IgM, Bioline Dengue Duo	44.5	100
			NS1, Dengue check combo	100	100
			IgM, Dengue check combo	77.7	50
			NS1,Dengue day 1	94.4	100
			IgM, Dengue day 1	27.8	65
			IgM Dengue duo cassette	61.1	95.1
Mahesh Reddy *et al..*[6]	Int J of Curr Micro App Sci	2016	NS1, SD Bioline	90.1	98.45
			IgM, Alere	92.57	98.5
M Moorthy *et al..*[7]	IJMM	2009	IgM, Dengue duo	81.8	75
Mohan Shukla *et al..*[8]	J Med Virol	2017	NS1, Dengue day 1	99.2	96
Pramiladevi R[9]	Sch J App Med Sci	2013	IgM, Dengue duo	69	86
Niloofa R *et al..* [10]	PLOS ONE	2015	IgM Leptocheck WB	86.1	84.5
Panwala T [11]	J Clin Diagn Res	2015	IgM Leptocheck WB	90.7	93.4
Eugene *et al..*[12]	BMC Infectious Diseases	2015	IgM Leptocheck WB	95	76.4
Goris MGA *et al..* [13]	PLOS Neglected Tropical Diseases	2013	IgM Leptocheck WB	78	98

In a high throughput laboratory such as ours, with a seasonal workload of about seventy five thousand samples during the rainy season (June – October) for the sero-diagnosis of Dengue and Leptospirosis, having a 24x7 availability of ELISA is the need of the hour. An accurate and timely diagnosis of Dengue and Leptospirosis is not only important for case management but also as a public health driving measure. An increased turn- around time leads to loss of patients to follow up. Most of the investigations are received during non-routine hours as per the flow of patients availing hospital services through the Fever OPD. The availability of only an interim report due to the use of rapid tests in non-routine hours, delays a confirmatory diagnosis by another day. This can create bottlenecks both at laboratory level as well as for the clinicians for tracing the final report. Considering the discordance rate between the rapids and ELISA, the GOI's directive of testing the samples by ELISA for confirmatory diagnosis of Dengue and Leptospirosis should be considered for seamless implementation.

CONCLUSION

This study tried to evaluate the performance of rapid tests for Dengue and Leptospirosis and also look at the feasibility of implementing ELISA based testing as a continuous platform. Where resources are limited, rapids can still be used until facilities and technical manpower for ELISA testing is available.

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