IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 9 Ver. VI (Sep. 2015), PP 26-32
www.iosrjournals.org

Diagnostic utility of saliva as non-invasive alternative to serum in suspected dengue patients.

[1]Dr A.Aparna, [2]Dr.R.Vinod Kumar, [3]Dr V.Muralikrishna,
[4]Dr.Rathod Pradeep Kumar.

[1.] Associate professor of paediatrics, [2]Assistant professor of paediatrics, [3]Professor of paediatrics
[4]Post graduate
[1,2,3,4]Department of Paediatrics , Niloufer Hospital, Osmania Medical College, Hyderabad.

Address for corresponding author: *Dr R. Vinod kumar, Assistant Professor, Department of paediatrics, Niloufer Hospital, Osmania Medical College, Hyderabad, Telangana State, India. Email:vkravilala @gmail.com*

Abstract:
Background: *Dengue viral infections are one of the most important mosquito borne diseases with around 100 million cases annually worldwide. The collection of blood samples is difficult during epidemics hence we attempted to find out the feasibility of saliva as an alternative, noninvasive, diagnostic test.*
Aim*: Detection of salivary NS1 and IgM/IgG antibody using rapid kit test for early diagnosis and comparing the feasibility and efficacy as an alternative to serum for early diagnosis of dengue infection.*
Settings and Design: *This is a evaluation of diagnostic test done in Niloufer Hospital For Women and Children, Osmania Medical College, Hyderabad,Telangana.*
Materials and methods: *88 patients of suspected dengue admitted during the period of June 2014 to October 2014 were tested with SD BIOLINE DENGUE DUO rapid immunochromatographic kit. Both Serum and saliva samples were tested simultaneously to detect both NS1antigen and IgM and IgG antibodies for dengue diagnosis.*
Results: *Of total 88 cases, dengue was positive in 52 serum samples and 46 salivary samples and negative in 36 serum samples and 42 salivary samples.*
Statistical analysis: *R Programming Software (Version 3.0.1) was used for Data Analysis. Sensitivity and specificity were calculated using epi R package. The serum RDT results were taken as quasi-gold standard and compared with the saliva RDT test results and the diagnostic efficacy was evaluated in terms of sensitivity and specificity. When NS1 antigen test result is taken separately-sensitivity is 86 % and specificity is 100 %,for IgM antibody sensitivity is 86% and specificity is 100% and for IgG antibody sensitivity is 88% and specificity is 100%.The sensitivity was 88.4 % and the specificity was 100% with salivary NS1, IgM and IgG all taken together.*
Conclusion: *saliva can be effectively used as an alternative to serum in diagnosis of dengue in community settings or in epidemic settings where venepunture in children would be a difficult task.*
Key words: *Dengue, Rapid diagnostic test, Saliva*

I. Introduction

Dengue viral infections are one of the most important mosquito borne diseases in the world. They may be asymptomatic or may give rise to undifferentiated fever, dengue fever, dengue hemorrhagic fever or dengue shock syndrome. Annually, 100 million cases of dengue fever and half a million cases of DHF occur worldwide.90 percent of DHF are children less than 15 years of age.

At present, dengue is endemic in 112 countries in the world [1, 2]. Early recognition and prompt initiation of appropriate treatment are vital to reduce disease related morbidity and mortality. Additional data about the disease leads to implementation or alteration in public health programs.

The total number of suspected dengue cases that presented to our hospital has increased phenomenally in the year 2014 as compared to the year 2012.since ours is pediatric centre and for such a case load collecting blood was difficult due to heavy work load, less manpower, child and parent anxiety and difficulties in vein puncture in children.

In the present study we have made an attempt to validate the salivary dengue rapid immunodiagnostic test under the conditions of routine community use and to investigate whether saliva could be a feasible alternative to serum as a non invasive technique for early diagnosis of dengue infection and early initiation of treatment.

II. Aim And Objective:

Detection of Salivary NS1, IgM and IgG antibody using rapid diagnostic kit test for early diagnosis of dengue fever in children and further comparing its feasibility and efficacy as an alternative to serum as a diagnostic tool for early detection of dengue infection.

III. Materials And Methods:

Study design: evaluation of diagnostic test a comparative study
Setting: Niloufer hospital for women and children, Osmania Medical College in Hyderabad, Telangana, India.
Study population: A total of 88 children between 3-14 years with suspected clinical features of dengue fever were included in the study.
Study period: June to October 2014.
Inclusion criteria:

1. All suspected dengue cases with fever more than 4 days in the pediatric age group 3 to 14 yrs were included in study.
2. Probable dengue – fever plus nausea/ vomiting, rash, body ache, tourniquet test positive, leucopenia.
3. Dengue with warning signs - Abdominal pain or Tenderness, Persistent vomiting, Clinical fluid accumulation, Mucosal bleed, Lethargy; restlessness, Liver enlargement >2cm, *Laboratory:* Increase in hematocrit concurrent with rapid decrease in platelet count
4. Dengue hemorrhagic fever and Dengue shock syndrome cases admitted in our hospital pediatric intensive care unit.
5. Serological positive i.e., Ns1 or IgM or /IgG cases diagnosed in private laboratories and referred to Niloufer hospital.
6. All cases sent for the microbiology lab for dengue serology.
7. Ultra sonogram of abdomen suggestive of dengue fever.

Exclusion criteria

1. Patients aged below 3 years and above 14 years.
2. Patients with routine laboratory testing suggesting other infectious disease bacterial /viral/other than dengue.
3. Patients whose saliva was not given on the same day with the serum samples.

Methodology

Approval was taken from the ethics committee of Osmania medical college. Informed consent was taken from parents/guardian. A detailed history and physical examination was done.

All patients were tested for dengue by SD BIOLINE DUO Dengue Ns1/IgM/IgG rapid test (immunochromatographic test) on both serum and saliva.

Dengue virus isolation, PCR analysis could not be done due to non availability of facility. Other laboratory investigations included hemoglobin, total and differential count, hematocrit, prothrombin time, activated partial thromboplastin time, liver transaminases, renal function tests, chest X ray, ultrasound abdomen.

Specimen collection and transport

Serum and saliva from patients suspected of dengue fever were simultaneously collected. Serum from patient was collected in plain vacutainer and saliva was collected in sterile plastic container. Test was performed with in half to one hour of collecting sample so no storage techniques or processing were done.
Testing of samples: Both the serum and saliva from the patient were simultaneously tested with the SD bioline rapid immunochromatographic test i.e, for Ns1, IgM and IgG as per manufacturer's instructions.
SD BIOLINE DENGUE DUO rapid test is an immunochromatographic one step assay designed to detect both dengue virus NS1 antigen and differential IgG or IgM antibodies to dengue virus in the serum ,plasma or whole blood. This kit contains 2 devices.
i.Left side contains Dengue NS1 antigen test.
ii.Right side Dengue IgG/IgM test.
i. Dengue NS1 antigen test: This test device contains a membrane strip which is pre coated with anti-Dengue NS1 antigen capture on test band region. The anti-dengue NS1 antigen colloid gold conjugate and serum plasma or whole blood sample move along the membrane chromatographically to the test region (T) and forms a visible line as the antibody-antigen-antibody gold particle complex forms.
ii. The dengue IgG/IgM rapid test on the right side is a solid phase immune chromatographic assay for rapid, qualitative and differential detection of IgG and IgM antibodies to the Dengue virus.
This test can detect all four serotypes of Dengue by using a mixture of recombinant dengue envelope proteins. This test is intended for the professional use to aid in the presumptive diagnosis between primary and secondary dengue infection. When compared to RTPCR this kit sensitivity and specificity was 92.4 % and 98.4%

respectively for Dengue NS1 antigen and 94.2% and 96.4% respectively for dengue IgM/IgG. It also had good correlation with Elisa with sensitivity and specificity of 94.2% and 96.4% respectively.

IV. Results And Observations

Total number of cases compared in the study using serum vs. saliva RDT was 88(table 1)

The mean age of the children included in the study is 7.31years with a mean standard deviation of 2.78 years. Total number of males was 51 (58%) and total number of females was 37(42%).

Table 1: Gender distribution among suspected dengue patients

Gender	Number	Percentage
Male	51	58%
Female	37	42%

Observations of dengue RDT on serum samples (table2)

Table 2: results of dengue RDT on serum

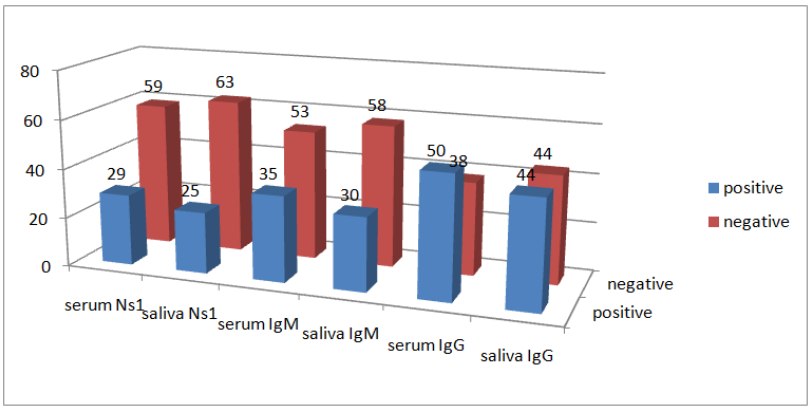
Serum RDT test	Cases negative (%)	Cases positive (%)	Total
Ns 1antigen	59(67%)	29(33%)	88
IgM antibody	53(60.2%)	35(39.8%)	88
IgG antibody	38(43.2%)	50(56.8%)	88

Observations of dengue RDT on salivary samples (table3)

Table 3: Results of dengue RDT on saliva

salivary RDT test	**Cases negative (%)**	**Cases positive (%)**	**Total**
Ns 1antigen	63(71.6%)	25(28.4%)	88
IgM antibody	58(65.9%)	30(34.1%)	88
IgG antibody	44(50%)	44(50%)	88

Fig 1: comparison of saliva and serum dengue RDT results



Statistical analysis

R Programming Software (Version 3.0.1) was used for Data Analysis. Sensitivity and specificity were calculated using epi R package.

The diagnostic efficacy was evaluated in terms of sensitivity and specificity that is calculated using a 2 × 2 cross-tabulation where a "gold standard" result (the peer-acknowledged, most accurate test) or reference standard result (normally, the test most widely used) is compared with the rapid test to determine diagnostic accuracy.

In the given study serum RDT results were taken as quasi-gold standard and compared with the saliva RDT test results.

Results of dengue Ns1antigen test on serum and saliva (table4).

Table 4: Dengue NS1 results of patients in serum and saliva

Test result NS1antigen	**Serum positive**	**Serum negative**	**Total**
saliva positive	25	0	25
saliva negative	4	59	63
Total	29	59	88

Results of dengue IgM antibody test on serum and saliva (table5).

Table 5: Dengue IgM test results of patients in serum and saliva

IgM antibody test	serum positive	serum negative	Total
saliva positive	30	0	30
saliva negative	5	53	58
Total	35	53	88

Results of dengue IgG antibody test on serum and saliva (table 6).

Table 6: Dengue IgG antibody results of patients in serum and saliva

IgG antibody Test	serum positive	serum negative	Total
saliva positive	44	0	44
saliva negative	6	38	44
Total	50	38	88

The overall result of the dengue test for NS1, IgM and IgG using saliva (table 7).

Table7: Overall Dengue test results in serum and saliva

Dengue (NS1+IgM+IgG)	serum positive	serum negative	Total
saliva positive	46	0	46
saliva negative	6	36	42
Total	52	36	88

The above results and observations were analyzed statistically and the following sensitivity and specificity pattern was obtained (table8).

Table 8: statistical analysis of salivary dengue tests as against serum samples

Test	Dengue Ns1	Dengue IgM	Dengue IgG	Dengue Ns1+IgM+IgG
Sensitivity% (confidence interval)	86.21(68.34-96.11)	85.71 (69.74-95.19)	88.00(75.69-95.47)	88.46(76.56-95.65)
Specificity% (confidence interval)	100 (93.94-100.00)	100 (93.28-100.00)	100 (90.75-100.00)	100(90.26-100.00)
Positive Predictive Value% (confidence interval)	100 (86.28-100.00)	100 (88.43-100.00)	100 (91.96-100.00)	100(92.29-100.00)
Negative Predictive Value% (confidence interval)	93.65(84.53-98.24)	91.38 (81.02-97.14)	86.36(72.65-94.83)	85.71(71.46-94.57)

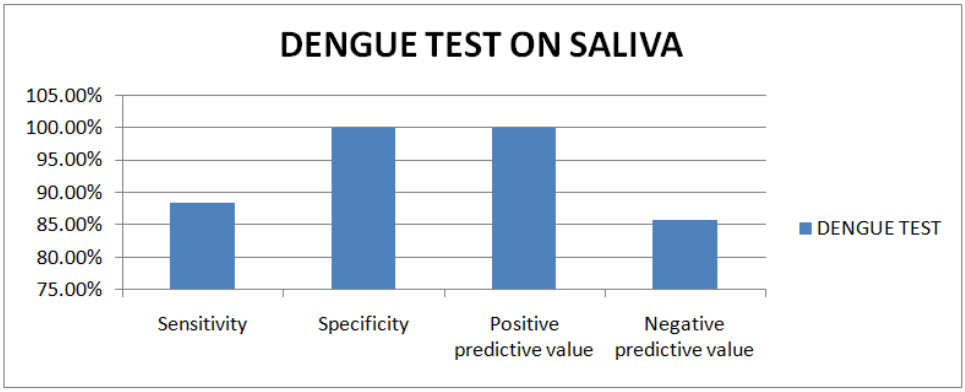


Fig 2: Statistical Representation of Dengue Test on Saliva

V. Discussion

The relationship of dengue with India has been long and intense. The first recorded epidemic of clinically dengue like illness occurred at madras (Chennai) in 1780[3].The first full blown epidemic of severe form of dengue occurred in north India in 1996[4]. Every year the epidemiology and clinical manifestations are changing and the incidence of cases has increased to the extent that it has become a major public health problem in our country.

The present study deals in comparing the rapid immunochromatographic card test results of salivary samples from suspected dengue patients with that of serum samples using the SD BIOLINE DENGUE DUO rapid diagnostic test kit and to screen out dengue cases as venous blood is difficult to collect and process, in a community based or remote settings or when sampling from young children.

In the given study saliva was chosen as sample because recently there is increasing appreciation that saliva reflects the normal internal characteristics and disease states of an individual. It may be due to its exchange with substances that compose the plasma. This occurs due to the presence of a thin layer of epithelial cells separating the salivary ducts from the systemic circulation, making it possible for substances to be transferred to the saliva through active carriage, diffusion through the membrane (ultra filtration), or through passive diffusion via a concentration gradient. Researchers are finding that saliva provides an easily available, noninvasive diagnostic medium for a rapidly widening range of diseases and clinical situations [5]. It has a simple and non-invasive collection method, is easy to store, and is inexpensive when compared to blood collection.

Research conducted in India on 80 patients suspected to have dengue and 25 control patients showed a good correlation between the diagnostics using saliva and the conventional test (through venous blood use), concluding that saliva has effective markers for dengue diagnosis.

The present study was done in the year 2014 from June to October. A total of 88 suspected cases of dengue were screened for both serum and saliva samples at the same time using the SD bioline dengue duo RDT kit and results were compared side by side.

This study is a unique study as are only few studies which have used saliva as sample for dengue diagnosis using IgM/IgG and also NS1 antigen and compared with serum RDTs.

Only balmaseda et al [6] had taken more number of cases (147) than the present study (88). In the present study primary dengue cases were only 2 and secondary dengue cases were 50 as ours is a tertiary care hospital where secondary dengue cases are referred from first referral units.

As most of the studies using saliva as specimen have applied ELISA method for comparing with serum and very few studies were done which have used RDT for comparison of test results, in this study sensitivity and specificity for different types of tests which were done on saliva as specimen for diagnosis of dengue are compared.

Balmaseda et al in 2003 used IgM capture Elisa technique to compare serum and saliva specimens of 147 cases. The serum and salivary IgM positive were 49% and 48.2% respectively where as in the present study it was 39.8% and 34.1%. Serum and saliva was negative in 51% and 51.8% respectively as compared to 60.2 % and 65.9% in this study.

The positivity rate for IgM in serum and saliva using ELISA was higher than the present study where RDT was used to detect IgM antibody in serum and saliva. The lower positivity rate in the present study could be attributed to the fever epidemic which was present during collection of cases. Viral fevers, malaria, other fevers would have contributed to the excess number of negative cases.

Cuzzobo et al [7] in 1998 had enrolled 35 patients. Total no of positive cases using gold standard (in-house ELISA, hemagglutination inhibition assay (HAI), or viral isolation.)was 24 and total number of cases positive with saliva used in this study was diluted with 1:4 dilution and stored at -80*Celsius until assayed was 22 with a Sensitivity of 92 %

Of the patients with dengue virus infection, 8 showed elevation of both salivary IgM and IgG (all secondary infections); 3 showed elevation of salivary IgM only (two primary infections and one secondary infection); 11 showed elevation of salivary IgG only (all secondary infections); and 2 with secondary infections were negative for both IgM and IgG.

None of the 11 patients with clinically suspected dengue and no laboratory evidence of infection produced a positive result in the saliva assay with a specificity of 100%.

Cuzzubo et al. had chosen Elisa technique for his study but the present study dengue rapid diagnostic kits were used for salivary diagnosis as well as serum. Positivity rate(68.5%) is more in cuzzubo et al when compared to present study(59%).Percentage of primary dengue patients in both studies are similar(100%).Percentage of Secondary dengue cases in both studies are also comparable(92% and 88%)

Chakravarti et al [8] 2007 conducted study on 80 patients using Elisa technique to compare the results of serum and saliva in suspected dengue patients. The sensitivity for IgM antibody in saliva was 100% as

compared to the present study which is 86% but the specificity in present study was 100% as compared to 70% in their study.

The sensitivity for IgG test in saliva is better (93.3%) compared to the present study (88%) and specificity in both studies was similar (100%).

In a study conducted by Ravi Banavar et al [9] in 2014, 40 patients were chosen of which 20 were serology positive and 20 were serology negative. The salivary samples of these patients were collected and subjected to dengue specific Elisa technique. There was no false positives diagnosed using saliva which suggests 100 percent specificity which is similar to the present study. The sensitivity was 100% as compared to 88% in the present study.

Table9: Comparision of sensitivity and specificity of dengue IgM of present study with other studies

Dengue IgM antibody	Present study IgM RDT	Balmaseda etal IgM elisa	Cuzzoboetal IgM elisa	Chakravarti et al IgM elisa	Banavar et al IgM elisa
Sensitivity	86%	90.3%	92%	100%	100%
Specificity	100%	92%	100%	70%	100%

The sensitivity of the present study is comparable with the other studies with confidence interval between 68% and 96%.In the study by Chakravarthi et al Salivary IgM antibodies were detected in 100% of the serum IgM-positive samples and in 30% of the serum samples that were negative for IgM antibodies whereas In the present study salivary IgM test was not positive in any of serum IgM negative samples. In the study done by Artimos de oliveira et al [10] in 1999 serum was diluted in 1:10 ratio and saliva was undiluted. The sensitivity on salivary IgM test was >80% when done after day 5 of fever. The specificity of the dengue IgM test in present study (100%) is better than that of balmaseda (92%) et al and chakravarti et al (70%). In the present study repeat sample was not taken as follow up. The repeat sample would have helped diagnose more number of dengue cases using saliva as specimen.

Table10: comparison of sensitivity and specificity of dengue IgG test results of present study with other studies

Dengue IgG antibody	Present study	Chakravarti et al	Cuzzubo et al
Sensitivity	88%	93.3%	86%
Specificity	100%	100%	100%
Positive predictive value	100%	100%	100%
Negative predictive value	86%	83.3%	78.5%

The sensitivity in the present study is lower than chakravarti et al but superior to cuzzubo et al. Dengue IgG antibody test helps in diagnosing primary dengue or secondary dengue and can be done on day 1 also.

Table11: dengue NS1 test results in saliva – chakravarti et al vs present study

Dengue NS1 antigen test	Present study	Shamala et al
Sensitivity	86%	65.41%
Specificity	100%	98.75%

There are only a few studies that have compared ns1 antigen using saliva as specimen.Ns1 antigen test using dengue duo test on saliva in the present study was positive in 25 of the 29 patients positive for serum ns1 showing a sensitivity of 86% and specificity of 100%. In the study by shamala devi et al [11] in 2009 in a total of 185 true dengue positive defined by virus isolation or positive reverse transciptase PCR or a raising trend in paired sample, Sensitivity for NS1 using SD bioline Ns1 antigen kit was 65.41% and specificity of 98.75% .The sensitivity and specificity of the present study is better than shamala et al

Limitations of the study

There was no gold standard test used to determine true positives and SD bioline dengue duo test on serum was taken as quasi gold standard and results were compared. Virus isolation, polymerase chain reaction, hemagglutination inhibition test, Elisa test were not done due to non availability of facilities.

VI. Conclusion

The detection of NS1/ IgM/ IgG in saliva would be preferable simple, on invasive bed side screening test as compared to serum for diagnosis of dengue fever in both endemic and non endemic areas but a negative result does not rule out dengue. Further studies are required to assess the performance and impact of early laboratory diagnosis of dengue in saliva using rapid diagnostic kits (SD bioline dengue duo) in the routine

clinical setting. As it is simple screening test, it can be even used in primary health centers without requirement of venepuncture. The salivary testing can also be done on day 1 of fever during dengue epidemics and does not require skilled personnel as it can be done even by peripheral health worker at any rural health centre.
The collection of saliva as a specimen for testing is a simple procedure and Children would cooperate rather than subjecting them to painful procedure of venepuncture.

VII. Summary

Total of 88 patients suspected of dengue admitted during the period of June 2014 to October 2014 were taken up in this study. Test was carried out to detect both NS1antigen and IgM and IgG antibodies in serum and saliva simultaneously using SD bioline dengue duo rapid immunochromatagrahic kit. RDT was taken as quasi GOLD standard as Elisa, hemagglutination inhibition test, rt PCR was not available. Of total 88 cases, dengue was positive in 52 serum samples and 46 salivary samples and negative in 36 serum samples and 42 salivary samples. The sensitivity was 88.4 %.and specificity was 100% ,when NS1 antigen test result is taken separately sensitivity is 86 % and specificity is 100 %, for IgM antibody sensitivity is 86% and specificity is 100% and for IgG antibody sensitivity is 88% and specificity is 100%. Thus saliva can be effectively used as an alternative to serum in diagnosis of dengue in community settings or in epidemic settings where venepunture in children would be a difficult task.

References

[1]. Pinheiro FP, Corber SJ. Global situation of dengue and dengue haemorrhagic fever and its emergence in the Americas. World Health Stat Q1997; 50:161–8.
[2]. World Health Organisation. Prevention and control of dengue and dengue haemorrhagic fever: comprehensive guidelines. WHO Regional publication, SEARO, No 29, 1999.
[3]. Narayanan M, Aravind MA, Thilothammal N, et al. Dengue fever epidemicin Chennai—a study of clinical profile and outcome. Indian Pediatr2002;39:
[4]. Kabra SK, Jain Y, Pandey RM, et al. Dengue haemorrhagic fever in children in the 1996 Delhi epidemic. Trans R Soc Trop Med Hyg 1999;93:294–8.
[5]. Mandel ID. The diagnostic uses of saliva. J Oral Pathol Med 1990;19(3):119-25
[6]. Angel Balmaseda et al Diagnosis of Dengue Virus Infection by Detection of Specific Immunoglobulin M (IgM) and IgA Antibodies in Serum and SalivaClinDiagn Lab Immunol. Mar 2003; 10(2): 317–322.
[7]. Andrea J. Cuzzubbo,1 David W. Vaughn,2,† Ananda Nisalak,2 Saroj Suntayakorn,3 JohnAaskov,4 and Peter L. Devine1, J ClinMicrobiol. Dec 1998; 36(12): 3737–3739. Detection of Specific Antibodies in Saliva during Dengue Infection
[8]. Chakravarti A1, Matlani M, Jain M.Immunodiagnosis of dengue virus infection using saliva.CurrMicrobiol. 2007 Dec;55(6):461-4. Epub 2007 Sep 27
[9]. Spoorthi Ravi Banavar1 and Vidya G.S.2Diagnostic Efficacy of Saliva For Dengue - A Reality in Near Future? A Piloting Initiative J ClinDiagn Res. Mar 2014; 8(3): 229–232.
[10]. Artimos de Oliveira S1, Rodrigues CV, Camacho LA, Miagostovich MP, Araújo ES, Nogueira RM. J Virol Methods. Diagnosis of dengue infection by detecting specific immunoglobulin M antibodies in saliva samples. 1999 Jan; 77(1):81-6.
[11]. Seok Mui Wang and Shamala Devi Sekaran. Early Diagnosis of Dengue Infection Using a Commercial Dengue Duo Rapid Test Kit for the Detection of NS1, IGM, and IGG.Am J Trop Med Hyg. Sep 2010; 83(3): 690–695.