IMMUNOLOGICAL RESPONSE TO DENGUE INFECTION AND DETECTED BY USING RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY TEST WITH POLYMERASE CHAIN REACTION FOR EARLY DIAGNOSIS

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ABSTRACT: **Mosquito-borne disease worldwide in tropical and sub-tropical regions Dengue (DNG-G) fever occurs. Even with dengue fever, which is also known as dengue hemorrhagic fever, blood pressure (shock) and sudden drop in death may occur. A positive PCR result is a definite proof of current infection and it generally confirms the infected serotype. A dengue fever vaccine, denguexia, currently has approved for use in people living in high incidence of dengue fever at age 9 to 45 years. The current review research acute phase is important for clinical care, implementing control measures, surveillance in diagnosis of dengue infection. Dengue fever was diagnosed by means of virus isolation, reverse transcriptase PCR or IgM and IgG based ELISA. Given the limitations of all the existing diagnostic methods, there is a need for rapid, sensitive and high throughput methods for detection of dengue virus in early stages of the disease. The review study was conducted with the objectives to evaluate a dengue virus NS1 antigen detection ELISA and a Taq Man based real time RT-PCR for detection of all six serotypes of dengue virus, as diagnostic tools for acute dengue virus infection.**

Keywords- Mosquito *Aedes aegypti*, Virus, Dengue, Hemorrhagic Fever, RT-PCR, Antibodies, denguexia

I.   INTRODUCTION

Mosquito acquires virus from feeding on infected persons blood- incubate virus for 8-10 days. Dengue transmitted to humans through bites of female *Aedes aegypti* mosquitoes. Virus circulates in blood of human for two to seven days. Found in tropical and sub-tropical areas worldwide, predominantly in urban and semi urban areas. A potentially lethal disease that affects 50 million a year four distinct but related viruses cause dengue. Recovering from one gives lifelong immunity to that strain, but not to the other three Flavavidrea, Chilunguniya and zika virus. Good evidence that further infection by different virus strains can lead to dengue hemorrhagic fever (DHF) a lethal complication of dengue. (Taia T. et, al 2017).

Dengue fever is caused by any one of four types of dengue viruses spread by mosquitoes that thrive in and near human lodgings. When a mosquito bites a person infected with a dengue virus, the virus enters the mosquito. When the infected mosquito then bites another person, the virus enters that person's bloodstream. After you've recovered from dengue fever, you have immunity to the type of virus that infected you but not to the other three dengue fever virus types. The risk developed severe dengue fever, also called as dengue hemorrhagic fever, actually infected a second, third or fourth time. About 2-.5 percent of DHF cases are fatal, with intensive supporting therapy, rate can drop to 1 percent but untreated it is as high as 20 percent. (Elizabeth A. et, al 2017)

1.1 Dengue fever mechanism

The Dengue virus contained RNA as genetic material and covered with capsid outer shell coated with protein enveloped and inner bilayer lipid. (fig.1) The mechanism behind that receptor mediated endocytosis fusion nucleocapsid release RNA replication translation budding furin cleavage progeny release (fig.2) in human cell and get decapitation with released of ssRNA bind with Endoplasmic reticulum were translated than proteolysis utillized Golgi apparatus release get mature and kick out from the cell.

1.2 Life cycle of *Aedes aegypti*

Aedes aegypti is characterized by complete metamorphosis insect with an egg, larvae, Pupae and adult stage. Mosquitoes lay their eggs on the inner, above the waterline of wet walls of containers with water 100 eggs at a time, hardy stick eggs bind with walls of a container like glue and also survived dried out for up to 8 months even over the winter in the southern United States. Female Mosquitoes attract towards very small amount of water for their life cycle. The responsible factors, materials, container storing water makes for a great "nursery." Beside that other bowls, cups, fountains, tires, barrels, vases shown in (fig 3.)
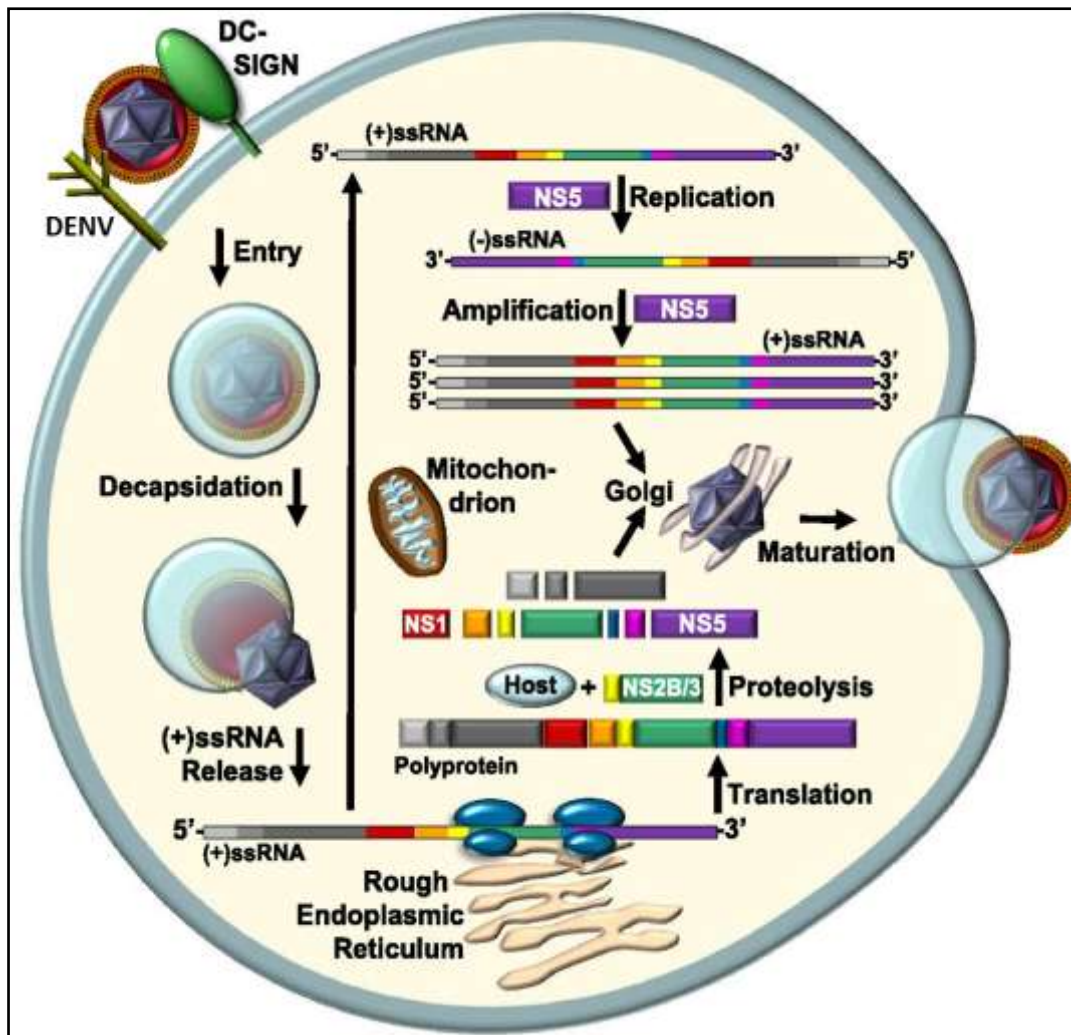
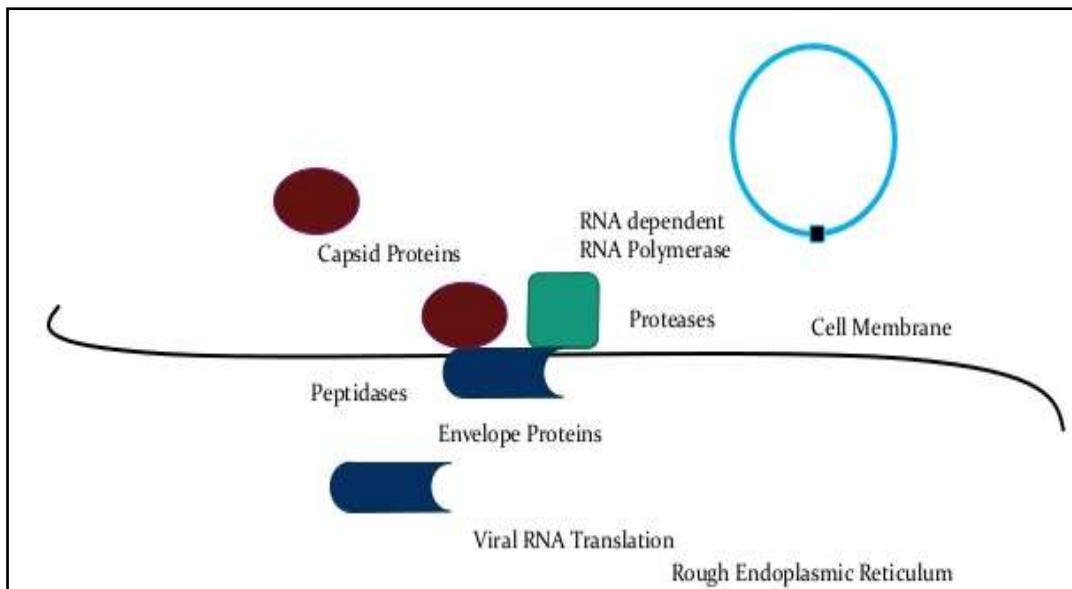**Fig. 1. Mode of Infection in Human cell**

Larva

Larvae emerged from eggs of female mosquito, but only after the water level rised to cover the eggs by rainwater or humans added water from the containers with eggs triggered emerged to the larvae. Microorganisms play a pivotal role for larvae feed on in the water. Three times molted, the larva becomes a pupa.
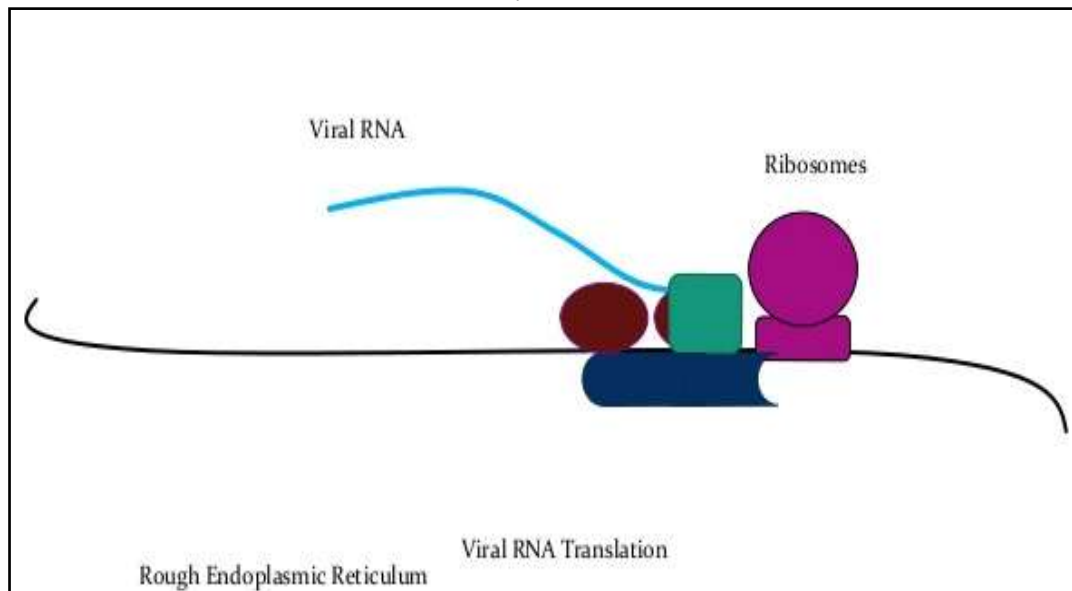
Pupa

Pupae develop until the body of the newly formed adult flying mosquito emerged from the skin pupal and leaves the water.
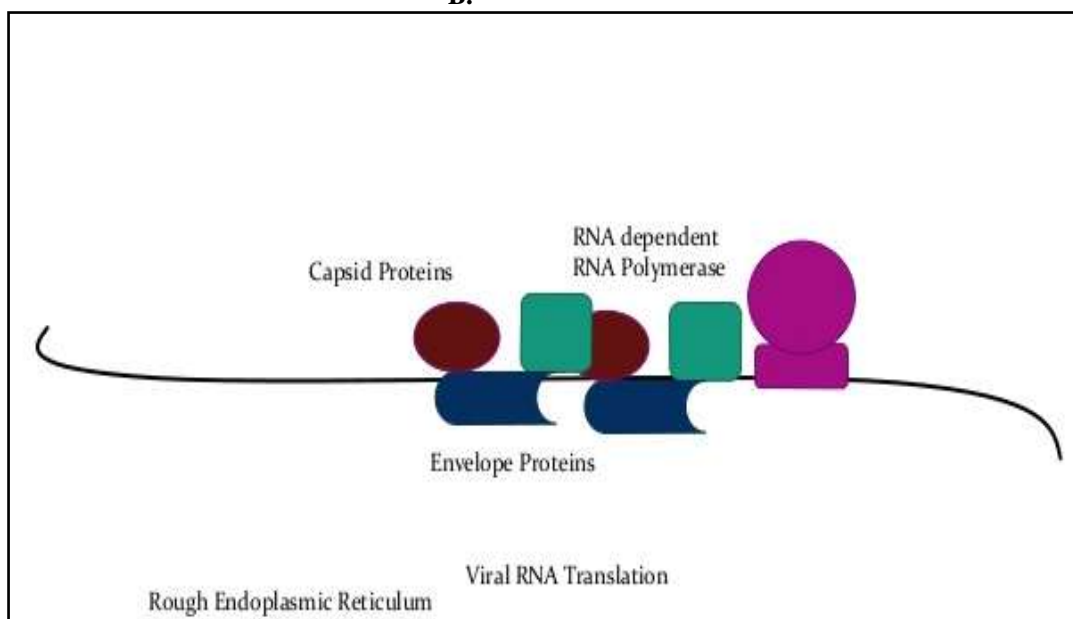
Adult

Adult female mosquitoes emerged after that the male mosquitoes feed on nectar from flowers and female mosquitoes feeded on humans and animals for blood to produce eggs. After feeded, female mosquitoes was look for water sources to lay more eggs and flies only a few blocks during its life, unlike other mosquito species, *Aedes aegypti* mosquitoes prefer to bite people. *Aedes aegypti* mosquitoes prefer to live near people. Founded inside buildings, homes and window are not used or doors are left propped opened.
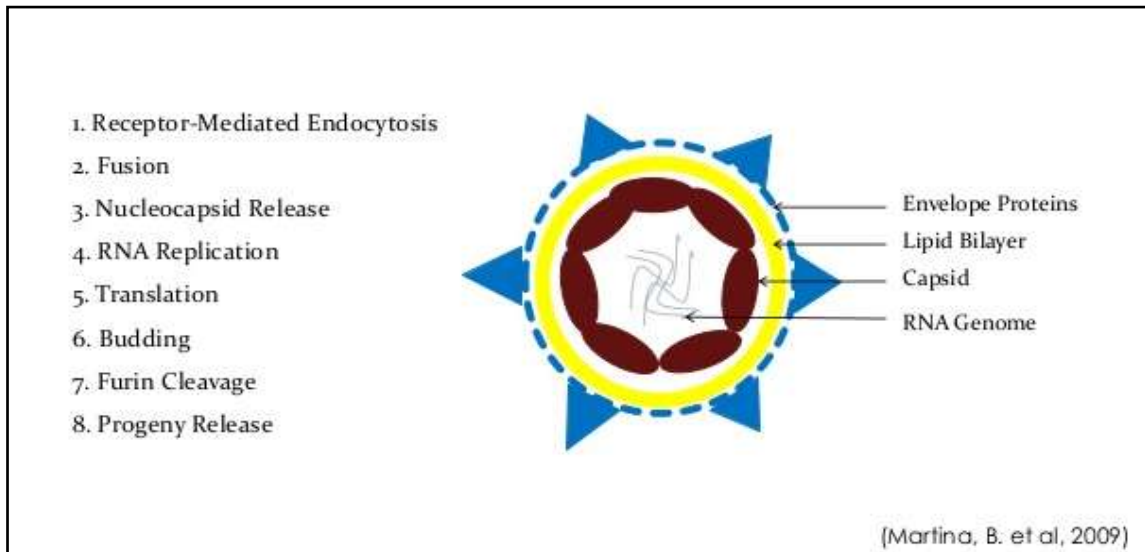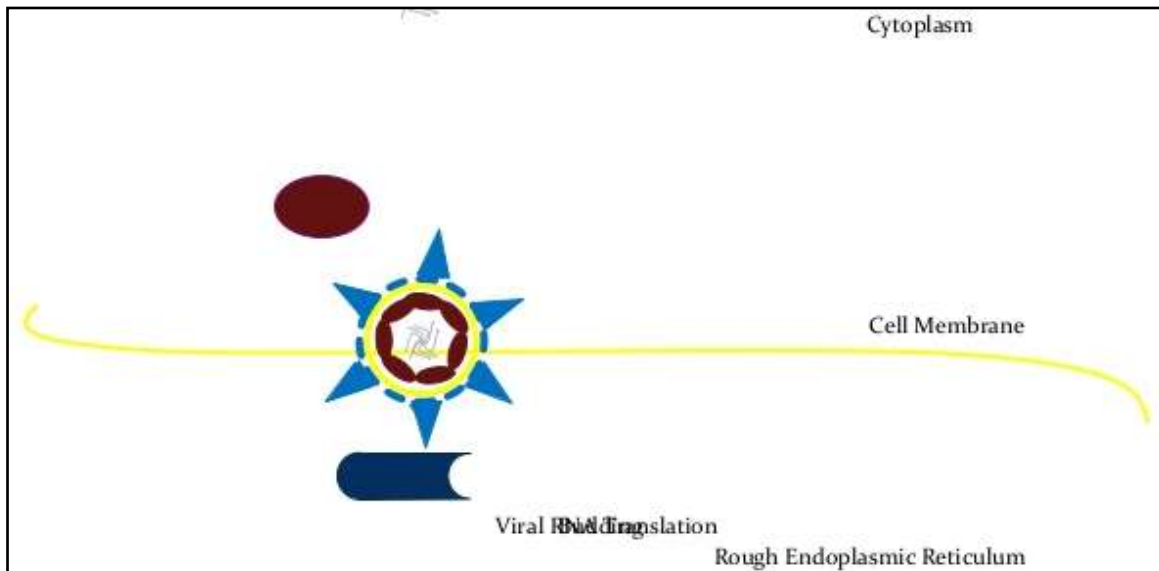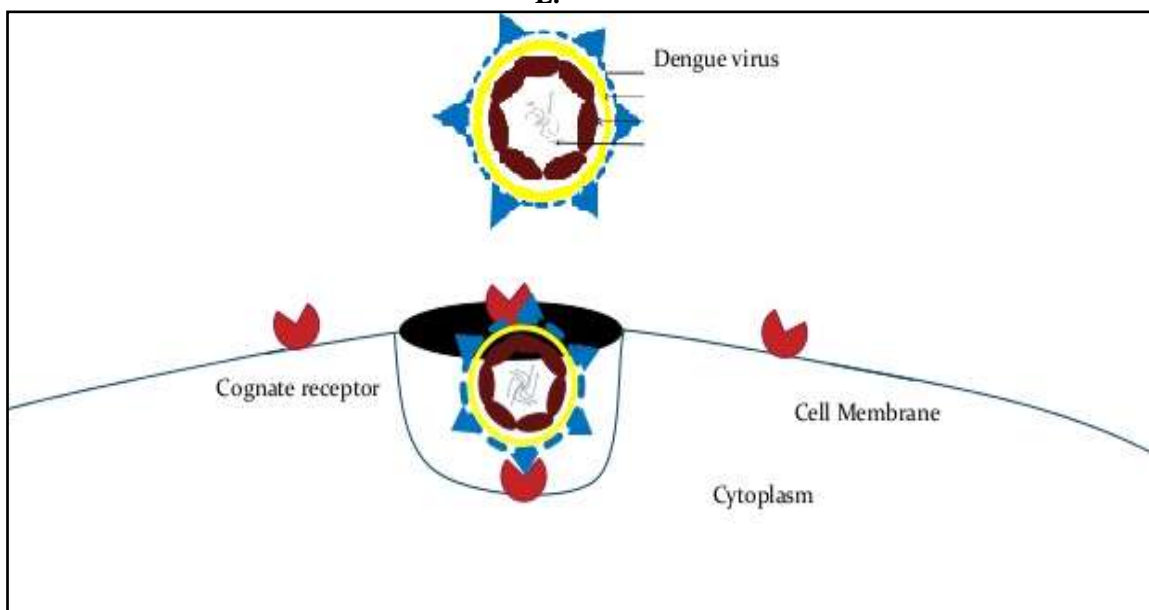
A.

B.

C.

1. Receptor-Mediated Endocytosis
2. Fusion
3. Nucleocapsid Release
4. RNA Replication
5. Translation
6. Budding
7. Furin Cleavage
8. Progeny Release

Envelope Proteins
Lipid Bilayer
Capsid
RNA Genome

(Martina, B. et al, 2009)

D.

Cytoplasm

Cell Membrane

Viral RNA Translation Budding

Rough Endoplasmic Reticulum

E.

Dengue virus

Cognate receptor

Cell Membrane

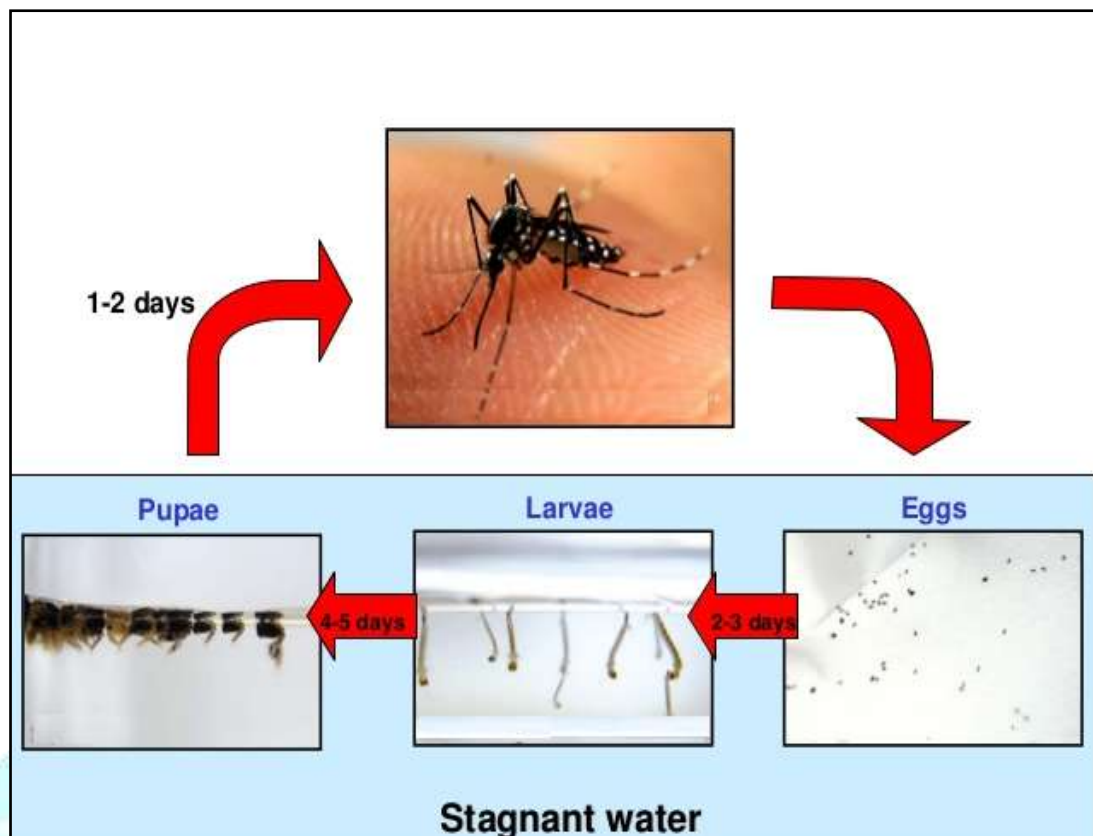Cytoplasm

F.
Fig 2. Mechanism of DENGUE Virus

Fig 3. Life cycle of *Aedes aegypti* Mosquito

The current review research acute phase is important for clinical care, implementing control measures, surveillance in diagnosis of dengue infection. dengue fever were diagnosed by means of virus isolation, reverse transcriptase PCR or IgM and IgG based ELISA. Given the limitations of all the existing diagnostic methods, there is a need for rapid, sensitive and high throughput methods for detection of dengue virus in early stages of the disease. The review study was conducted with the objectives to evaluate a dengue virus NS1 antigen detection ELISA and a TaqMan based real time RT-PCR for detection of all six serotypes of dengue virus, as diagnostic tools for acute dengue virus infection.

II.   RESEARCH METHODOLOGY

The acute phase serum samples of patients (n=153) presenting with dengue fever were subjected to NS1 antigen detection and real time RT-PCR. The results were compared to those of virus isolation in the C6/36 cell lines (n=55). (Ahmed NH, Broor S, 2014)

2.1 Symptoms

Symptoms or no signs during a mild case of dengue fever several people, especially children and teens, may experience. Symptoms were occurred, usually from four to seven days after people were bitten by an infected mosquito. Dengue fever causes a high fever at 104^0 F degrees and at least two of the symptoms are Headache, Muscle, bone and joint pain, Nausea, Vomiting, Pain behind the eyes, Swollen glands, Rash. Most people recovered within a week or more, rather than some cases, symptoms worsen and can become life-threatening. Blood vessels often become damaged and leaky. As well as the number of cells were clot-formed (platelets) in bloodstream drops; cause a severe form of dengue fever, also known as dengue hemorrhagic fever, severe dengue or dengue shock syndrome.
Symptoms and Signs of dengue hemorrhagic fever a life-threatening emergency for people infected, Severe abdominal pain, Persistent vomiting, Bleeding from your gums or nose, Blood in your urine, stools or vomit, Bleeding under the skin, which might look like bruising, Difficult or rapid breathing, Cold or clammy skin (shock),Fatigue, Irritability or restlessness

2.2 Diagnosis of Dengue

Dengue can be diagnosed by isolation of the virus, by serological tests method, or by used molecular biotyping methods. Diagnosis of recent dengue infection established by testing serum samples during the first 6 days of early convalescent phase. Acute infection with dengue virus is confirmed when the virus is isolated from autopsy tissue specimens or serum, or by reverse

transcription-polymerase chain reaction (RT–PCR) to find out the specific dengue virus genome is identified from serum or plasma, cerebrospinal fluid, or autopsy tissue specimens during an acute febrile illness shown in (fig.4)
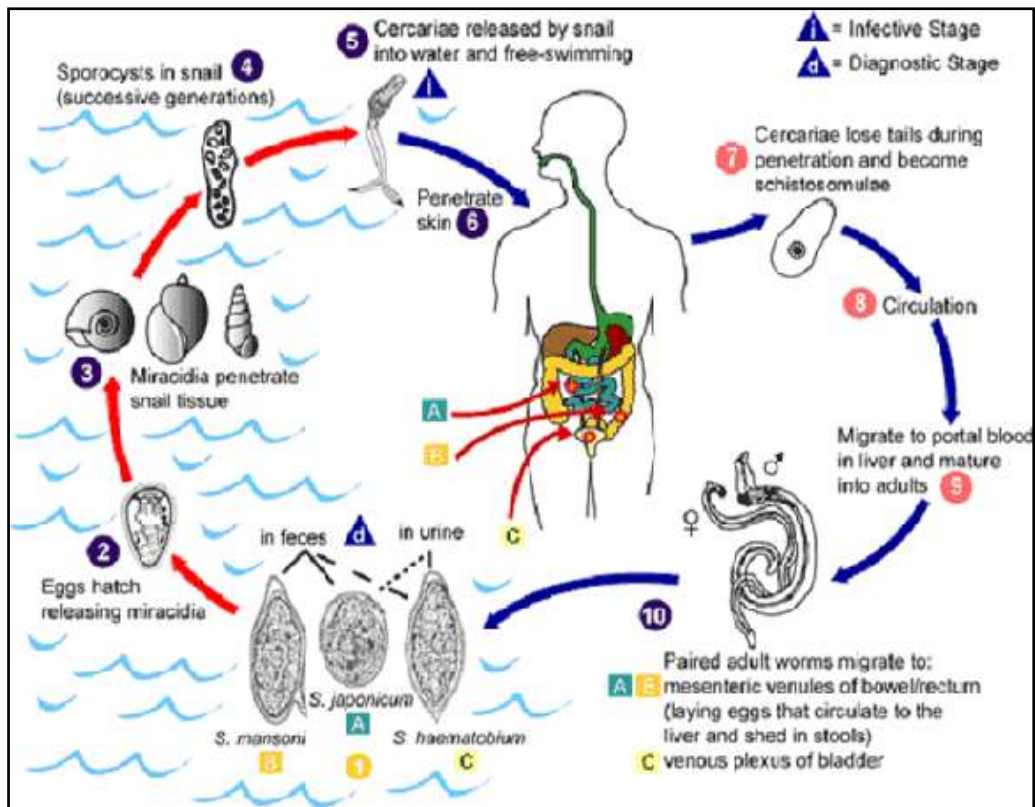

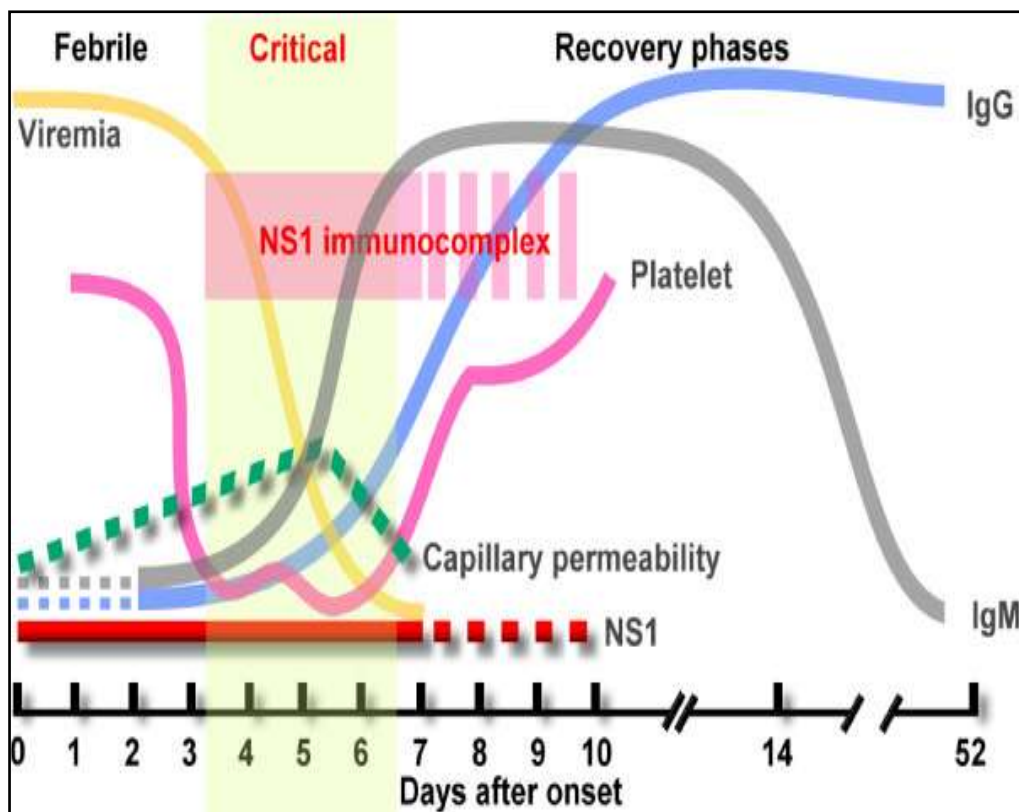**Fig 4. Infected stage of human and their Diagnostic stages**

Fig. 5. NS1 Immunocomplex with serum

Methods such as one-step, real time RT–PCR or nested RT–PCR are now widely used to detect dengue viral genes in acute-phase serum samples.

This detection coincides with the viremia and the febrile phase of illness onset. Acute infections can also be laboratory confirmed by identification of dengue viral antigen or RNA in autopsy tissue specimens by immunofluorescence or immunohistochemical analysis, or by seroconversion from negative to positive IgM antibody to dengue or demonstration of a fourfold or greater increase in IgG antibody titers in paired (acute and convalescent) serum specimens. (fig.5 & 6) (Taia T. et, al 2017).
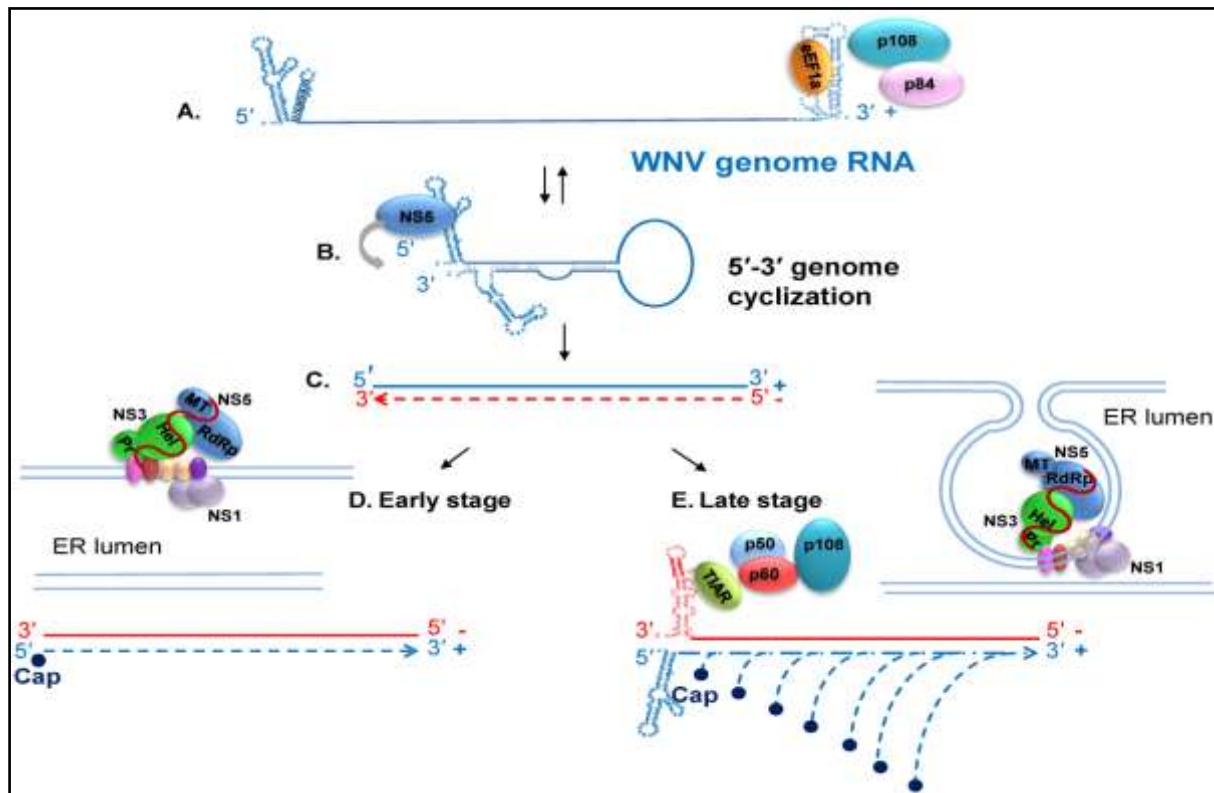

Fig. 6. Whole Network Genome of Virus cyclization

Patients who have IgM antibodies to dengue for detection in their serum specimen via an IgM antibody capture by enzyme-linked immunosorbent assay (MAC-ELISA) and had either and negative RT–PCR result in the acute phase specimen).[1] This is due to the fact that IgM antibodies for dengue may remain elevated for 2 to 3 months after the illness. The elevated IgM observed in a sample could be the result of an infection that occurred 2 to 3 months ago. In this review we have taken the past history patient's medical report, vaccination record and recent travel history, (especially yellow fever vaccination) to determine the likelihood that the current acute febrile illness were due to an infection with dengue virus. (Taia T. et, al 2017). with suspected dengue infection patient submits a late acute phase specimen that is negative (e.g., by RT–PCR and MAC-ELISA), and they do not submit a convalescent specimen, they are classified as a laboratory-indeterminate case.

2.3 Immunological Response to Dengue Infection

The acquired immune response following a dengue infection consists of the production of IgM and IgG antibodies primarily directed against the virus envelope proteins. The immune response varies depending on whether the individual has a primary (first dengue or other flavivirus infection) versus a secondary (had dengue or other flavivirus infection in past) dengue infection. In general, diagnosis of dengue is dependent on the phase of the infection. The general timeline of a primary infection from virus isolation or identification, to IgM detection followed by IgG detection is as follows (Taia T. et, al 2017).

A primary dengue infection is characterized by a slow and low titer antibody response. IgM antibody is the first immunoglobulin isotype to appear. Anti-dengue IgG is detectable at low titer at the end of the first week of illness, and slowly increases. In contrast, during a secondary infection, antibody titers rise extremely rapidly and antibody reacts broadly with many flaviviruses. High levels of IgG are detectable even in the acute phase and they rise dramatically over the preceding two weeks. The kinetics of the IgM response is more variable. IgM levels are significantly lower in secondary dengue infections and thus some anti-dengue IgM false-negative reactions are observed during secondary infections. According to the Pan American Health Organization (PAHO) guidelines 80% of all dengue cases have detectable IgM antibody by day five of illness, and 93-99% of cases have detectable IgM by day six to ten of illness, which may then remain detectable for over 90 days. (Taia T. et, al 2017and PAHO/WHO 2017).

MAC-ELISA has become an important tool for routine dengue diagnosis, MAC-ELISA has a sensitivity and specificity of approximately 90% and 98%, respectively but only when used five or more days after onset of fever (i.e., in convalescent phase). Different formats such as capture ELISA, capture ultramicroELISA, dot-ELISA, AuBioDOT IgM capture and dipsticks have

been developed. Serums, blood on filter paper, and saliva (but not urine) are useful for IgM detection if samples are taken in convalescent phase of illness (Vasquez et al., 2006). A variety of different commercial kits is available with variable sensitivity and specificity. Dengue diagnosis becomes even more challenging because dengue IgM antibodies also cross-react to some extent with other flaviviruses such as JEV, SLE, WNV and YFV. (fig.7& 8) (Taia T. et, al 2017).
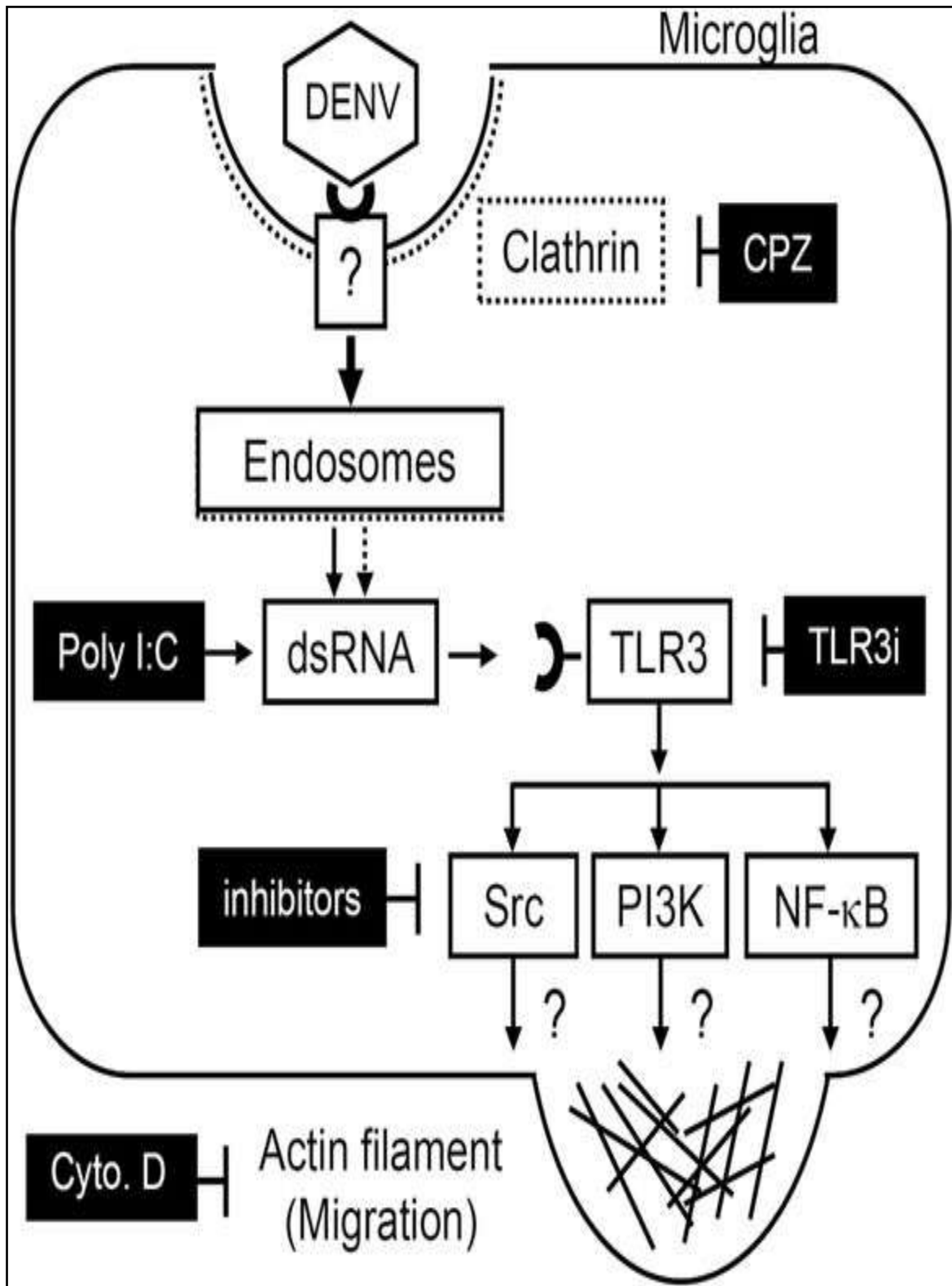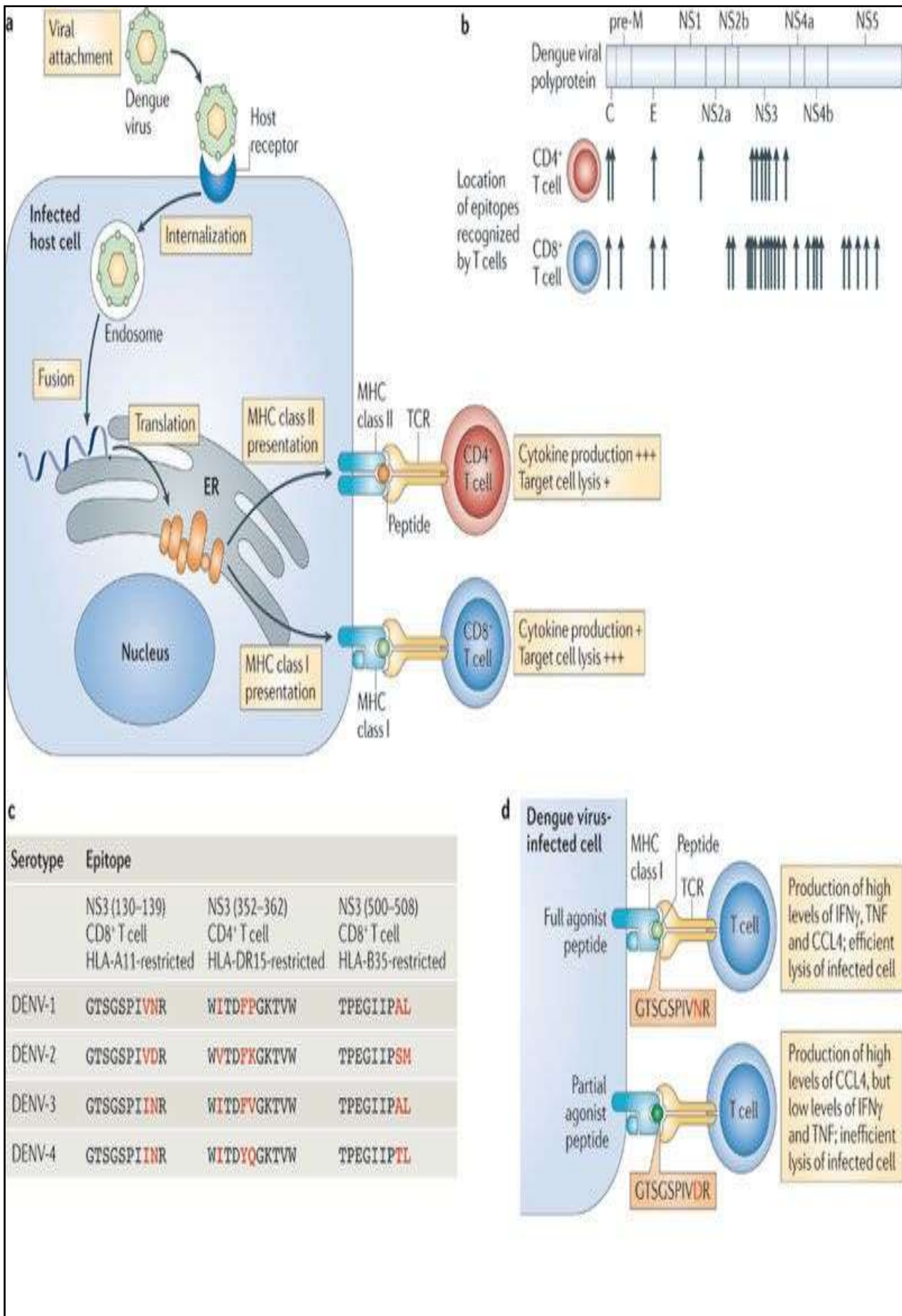

Fig. 7. Mode of action of DENV

Fig.8. Immunological Response to Dengue Infection *DENV-reactive IgG,DENV-reactive IgM,Dengue viral protein, NS1*

III.   RESULT AND DISCUSSION

DENV can be detected in the blood (serum) from patients for approximately the first 5 days of symptoms. Currently, several PCR tests are employed to detect the viral genome in serum. [6] In addition, virus can be isolated and sequenced for additional characterization. Real time RT–PCR assays have been developed and automated; but none of these tests are yet commercially available. Because antibodies are detected later, RT–PCR has become a primary tool to detect virus early in the course of illness.(Table 1) Current tests are between 80-90% sensitive, and more that 95% specific.(fig. 9) A positive PCR result is a definite proof of current infection and it usually confirms the infecting serotype as well. However, a negative result is interpreted as "indeterminate". Patients receiving negative results before 5 days of illness are usually asked to submit a second serum sample for serological confirmation after the 5th day of illness (Kukreti H,. et,al 2006).
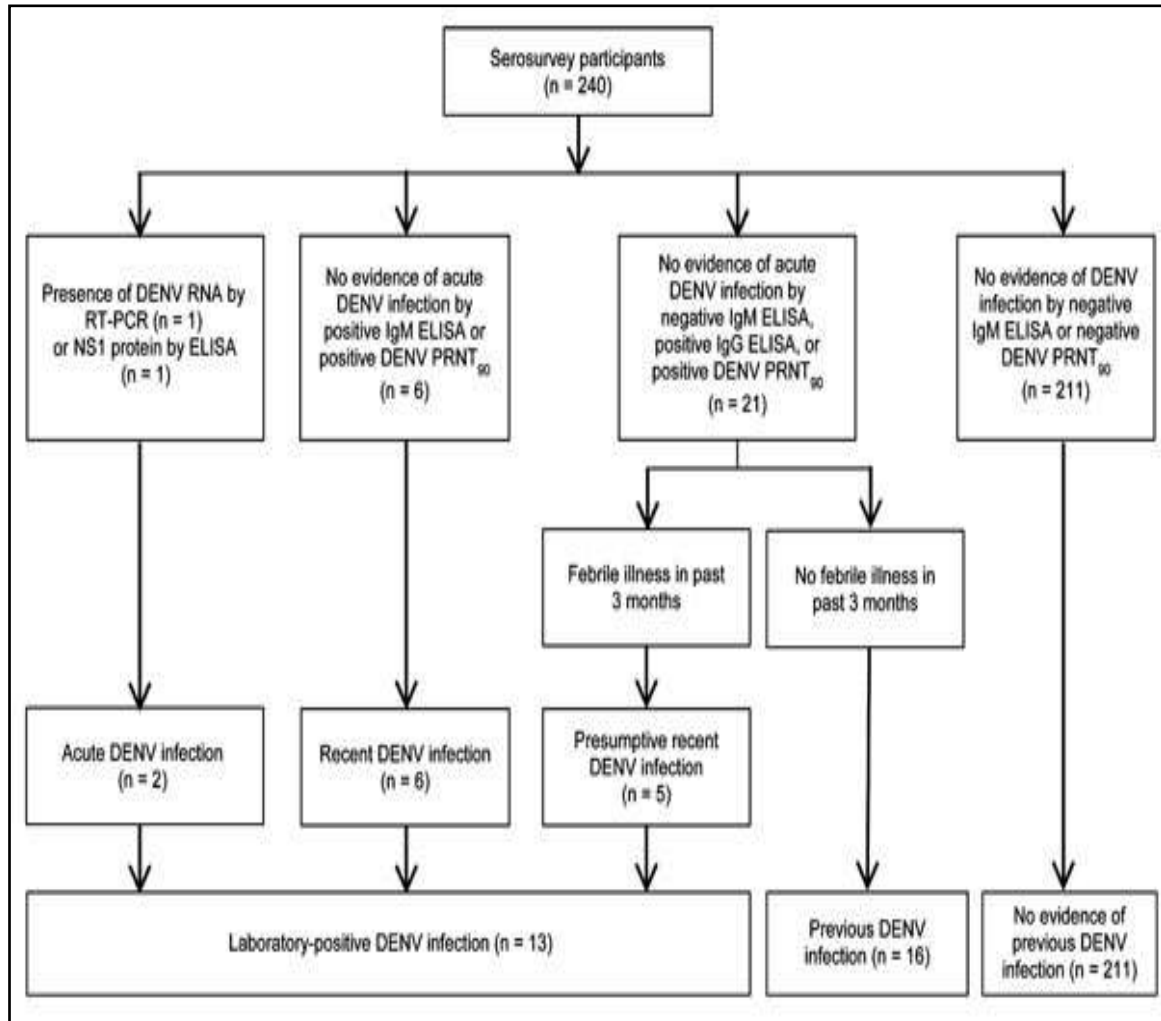

Fig.9 Conceptual Frame work of DENV

3.1   Testing Algorithms for Dengue

3.1.1 Sample for Diagnosis

Types of Samples	Interval since date of onsets of symptoms	Types of Analysis
Acute	Upto 5 weeks	RT-PCR
Convalescent	6 or more days	Serology

3.2 PCR (Polymerase Chain Reaction)

Quantitative PCR for example, can be used to quantify and analyze single cells, as well as recognize DNA, mRNA and protein confirmations and combinations. The DEN-1showed that all band were in single lane but lane 1 & 2 at 375 bp and 171 bp were separated long using forward and reverse primer under agarose gel electrophoresis beside that DEN-2,DEN-3,DEN-4 band were fall on 171 bp in common lane showed the significant of highly conserved sequence and semi conservative.(fig. 10& 11)
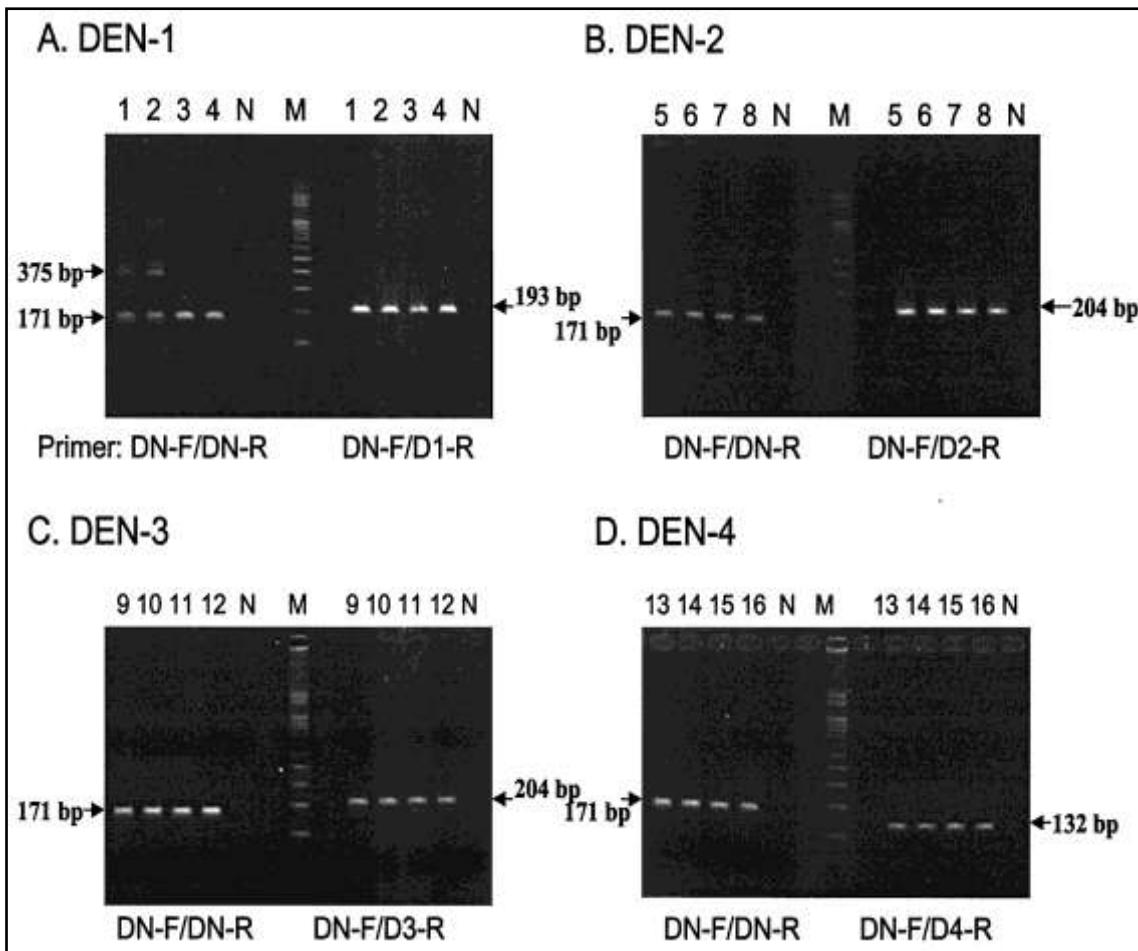
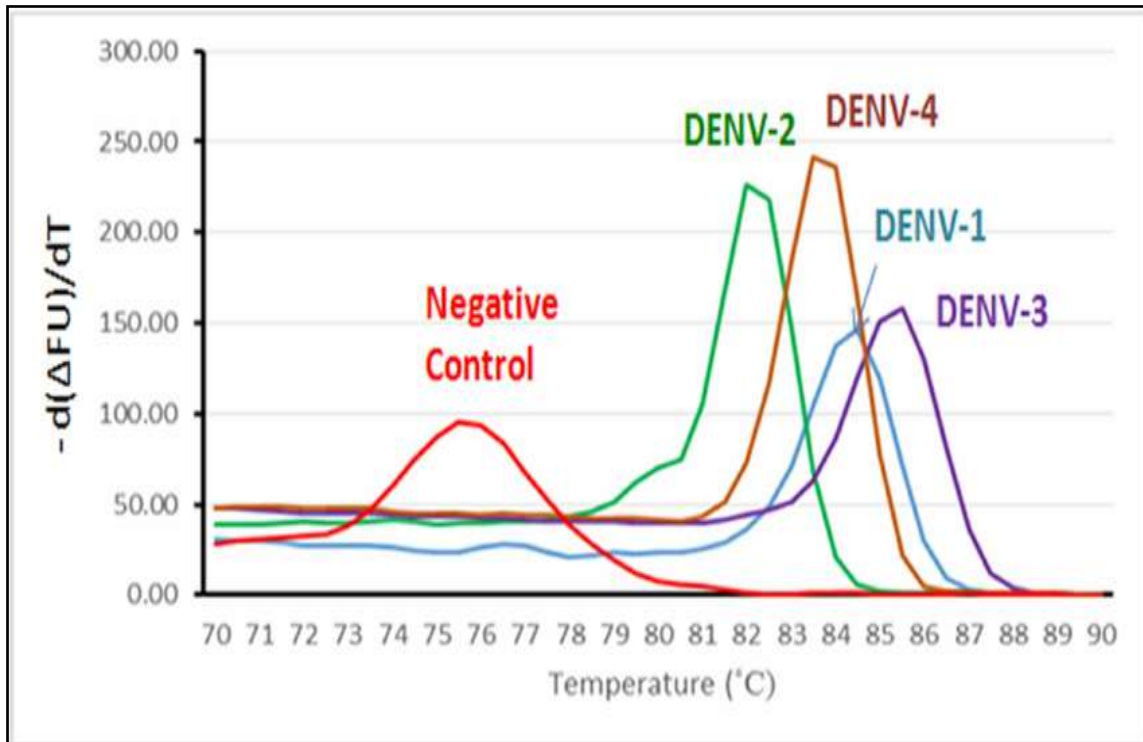**Fig. 10. PCR amplification on Agarose gel electrophoresis**

Fig. 11. Comparison between all DENV

3.2   MAC ELISA

Clinical suspected case of dengue the IgM antibody capture ELISA (MAC-ELISA) most commonly employed in diagnostic laboratories and commercial available diagnostic kits. The assay is based on capturing human IgM antibodies on a microtiter plate using anti-human-IgM antibody followed by the addition of dengue virus specific antigen (DENV1-4). The antigens used for this assay are derived from the envelope protein of the virus. (fig. 2.) One of the limitation of this testing is the cross reactivity between other circulating flaviviruses. This limitation must be considered when working in regions where multiple flaviviruses co-circulate. IgM detection is not useful for dengue serotype determination due to cross-reactivity of the antibody (Dar L,2003). (fig. 13)
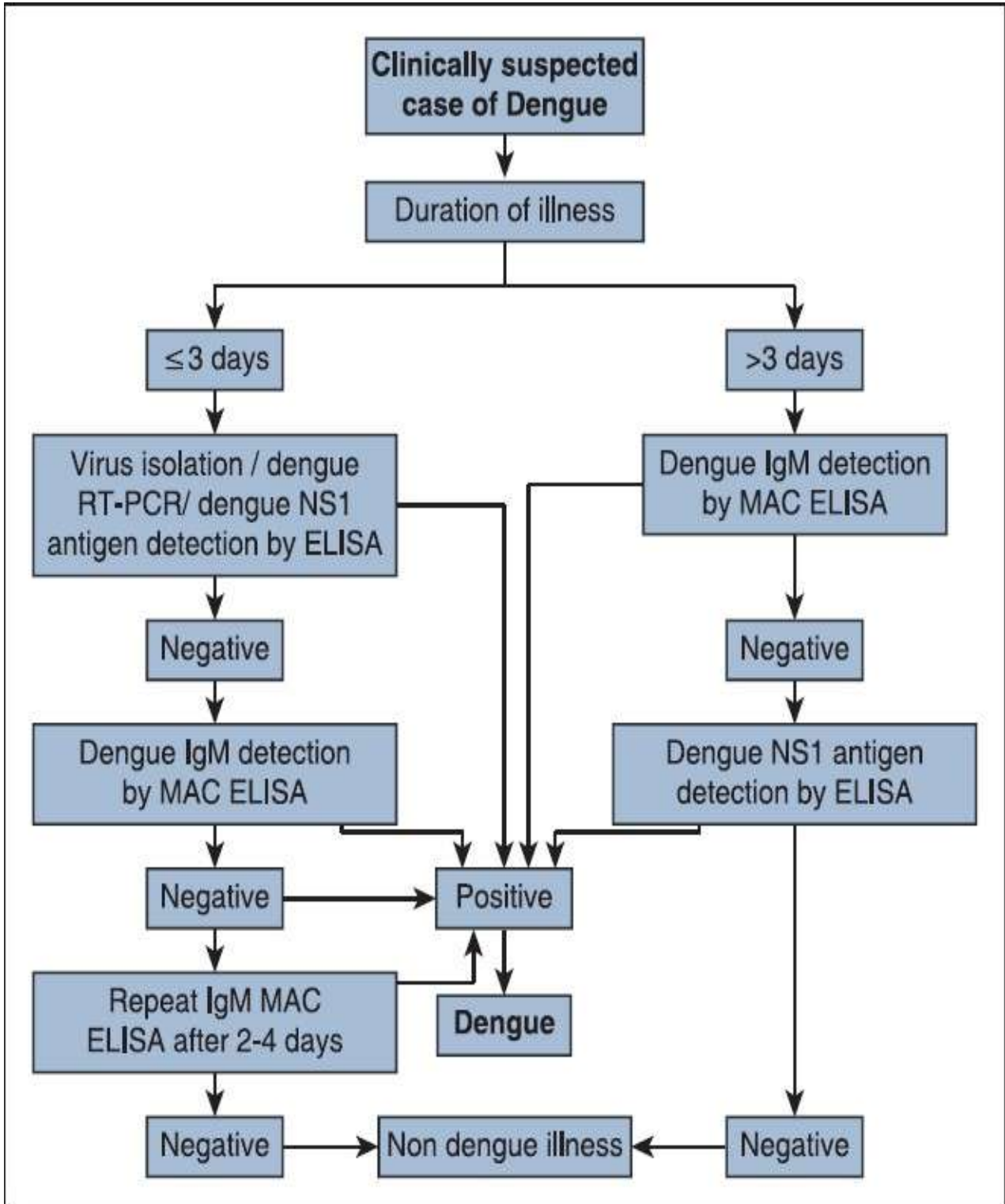

Fig. 12 Mechanism and mode of action of MAC ELISA against the Suspected Case of Dengue
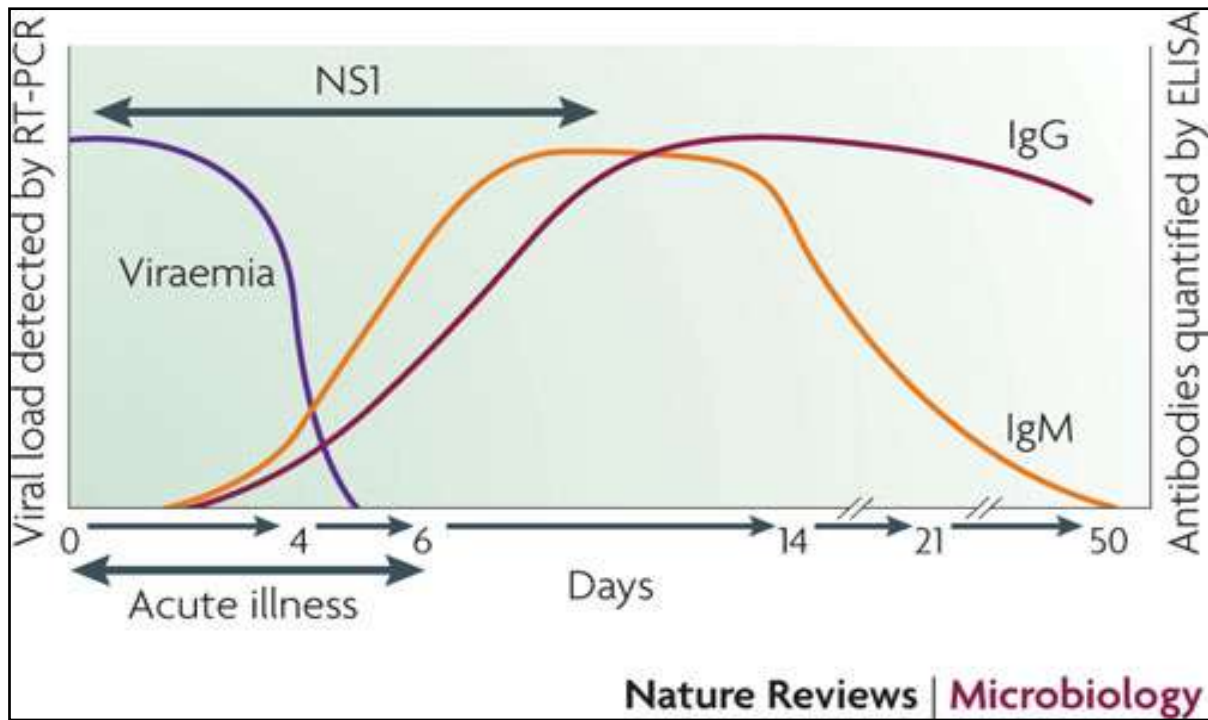
Fig. 13 Viral load detected by RT PCR in Case of NS1 realtion with Antibodies quantified by ELISA

3.3 IgG ELISA

The IgG ELISA used for the detection of a past dengue infection utilizes the same viral antigens as the MAC ELISA. This assay correlates with the hemagglutination assay (HI) previously used. In general IgG ELISA lacks specificity within the flavivirus serocomplex groups. Primary versus secondary dengue infection can be determined using a simple algorithm. Samples with a negative IgG in the acute phase and a positive IgG in the convalescent phase of the infection are primary dengue infections. Samples with a positive IgG in the acute phase and a 4 fold rise in IgG titer in the convalescent phase (with at least a 4, 6, 14 21 and 50 acute illness with 7 days day interval between the two samples) is a secondary dengue infection. **(fig.13)**

3.4 NS1 ELISA

The non-structural protein 1 (NS1) of the dengue viral genome has been shown to be useful as a tool for the diagnosis of acute dengue infections. [4] Dengue NS1 antigen has been detected in the serum of DENV infected patients as early as 1 day post onset of symptoms (DPO), and up to 18 DPO.(fig.13) The NS1 ELISA based antigen assay is commercially available for DENV and many investigators have evaluated this assay for sensitivity and specificity. The NS1 assay may also be useful for differential diagnostics between flaviviruses because of the specificity of the assay.[6]

3.5 PRNT (Plaque Reduction and Neutralization Test)

Plaque Reduction and Neutralization Test (PRNT) and the microneutralization PRNT can be used when a serological specific diagnostic is required, as this assay is the most specific serological tool for the determination of dengue antibodies. (fig.14) The PRNT test is used to determine the infecting serotype in convalescent sera. This assay measures the titer of the neutralizing antibodies in the serum of the infected individual and determines the level of protective antibodies this individual has towards the infecting virus. The assay is a biological assay based on the principle of interaction of virus and antibody resulting in inactivation of virus such that it is no longer able to infect and replicate in cell culture. Some of the variability of this assay is differences in interpretation of the results because of the cell lines and virus seeds used as well as the dilution of the sera.
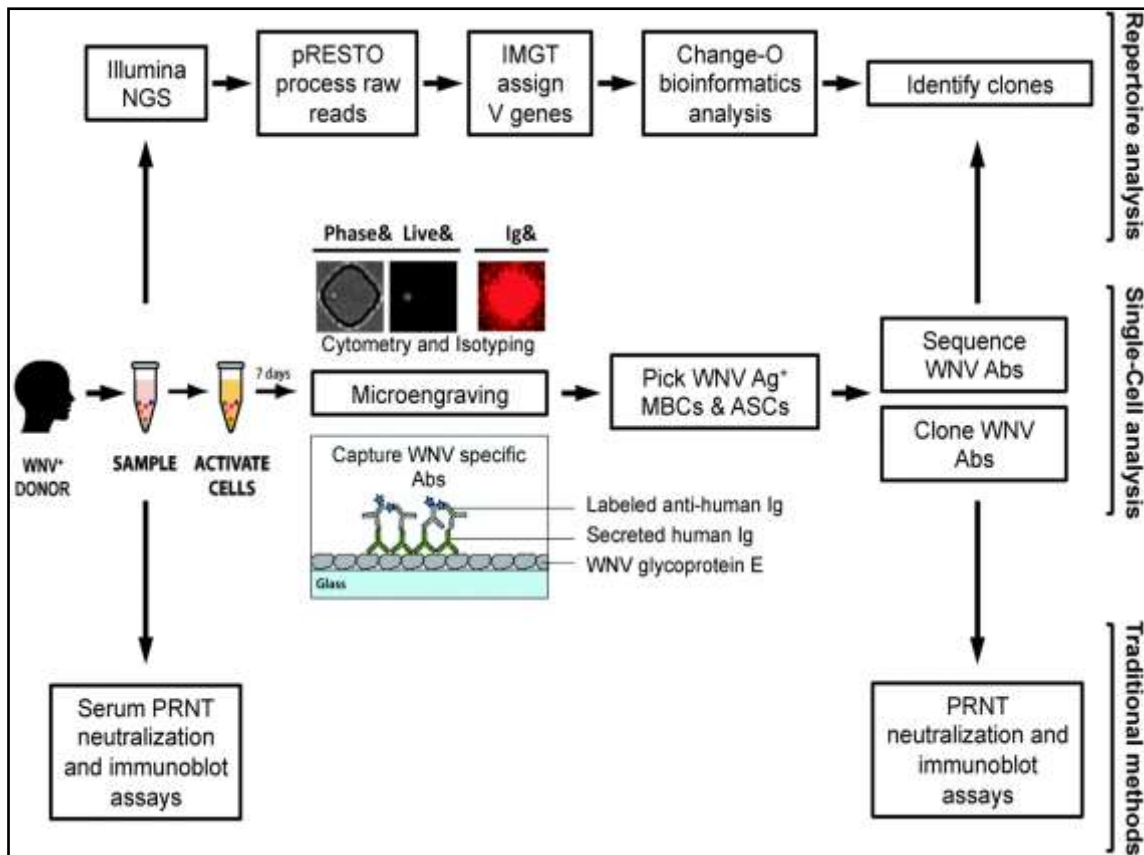
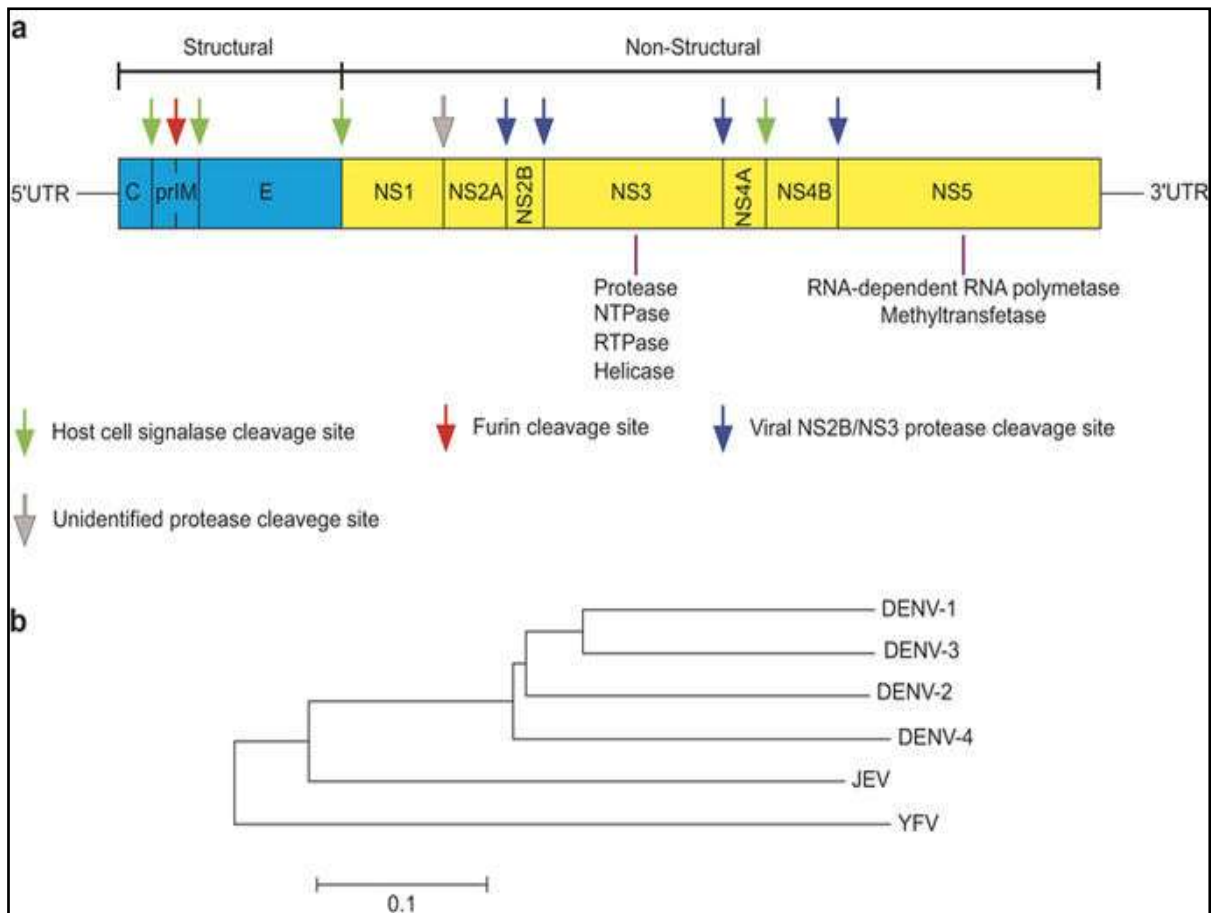Fig. 14 Serum Plaque reduction and neutralization test of suspected dengue patients

Fig. 15. RT PCR confirmed symptomatic cases

After RT PCR result the structural and Non structural gene were fin out in the suspected case of dengue. The structural host cell signalase cleavage site in it but in the case of Non structural they are RNA- dependent RNA polymerase Methyltransfesate at 3 prime of the gene. there are many enzymes worked protease, NTPase,RTPase, Helicase at N33 site. After gene amplified construction of phylogenetic tree with the help of UPGMA with PHYLIP. The dendrogram show the similarity between the DENV in suspected dengue DENV-1 and DENV-3 were gave 100 % similarity among the species but 60 % dissimilar against the rest of them. DENV-2 is 50 % similar and 50 % dissimilar, DENV-4 has showed 65 % similar to0 all and 35 % dissimilar among rest of them, JEV is showed 80 % dissimilar and YFV showed 100 % dissimilar among all the viruses at phylogenetic cluster. values of NS1 Ag detection ELISA showed positive , negative predictive, specificity, sensitivity, efficiency were 73.6, 83.5, 100, 100 and 72% respectively while for real time RT-PCR these were 77.3, 77.4, 100, 100 and 76% respectively. sensitivity of NS1 showed maximum and real time RT-PCR in three days of fever was seen in two days of fever and that of antigen detection ELISA

IV. CONCLUSION

One dengue fever vaccine, Dengvaxia, is currently approved for use in those ages 9 to 45 who live in areas with a high incidence of dengue fever. The vaccine is given in three doses over the course of 12 months. Dengvaxia prevents dengue infections slightly more than half the time. Detection of dengue with the help of NS1 antigen detection ELISA and real time RT-PCR were found to be fast, convenient and efficient tests for diagnosing of dengue fever in acute phase and the diagnosis could be made as early as within three days of onset of fever.

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