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Introductory Chapter: Serum Components as Rapid Diagnostic Biomarkers During Flavivirus Infection

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1. Introduction

Infectious diseases are closely dependent on the nature and complexity of human behaviour, as they reflect who we are, what we do and how we interact with other people, animals and the environment. Worldwide, these diseases remain a leading source of human morbidity and mortality in all age groups [1]. Their emergence is thought to be driven largely by socio-economic, environmental and ecological factors [2]. According to Centre for Strategic and International studies, globally 16% of the deaths each year are from infectious diseases, this number not only causes huge burden but also produces immense challenge toward building better healthcare system. The past few years witnessed emergence and re-emergence of several microbial infections. A majority of them have been vector-borne infections (especially, the arthropod-borne) viral diseases, which constitute 90% of the re-emerging diseases [3]. Mosquito-borne *Flaviviruses* provide some of the most important examples of emerging and resurging diseases of global significance [4]. Virulent strains of these viruses are continually evolving and expanding their geographic range, thus rapid and sensitive screening assays are required to detect emerging viruses and monitor their prevalence and spread. Diagnostic tests play a major role in the clinical care of patients with infectious diseases, including detection of specific pathogens, discovery of new pathogens, determining appropriate therapy, monitoring response to therapy, assessing prognosis and disease surveillance. Whether caring for an individual patient with an infectious disease or responding to a worldwide pandemic, the rapid and the accurate establishment of a microbial cause is fundamental to quality care [5].

2. Flaviviruses

Flaviviruses are responsible for a number of important mosquito-borne diseases of man and animals globally. These are a highly diverse group of RNA viruses classified within the genus *Flavivirus*, family *Flaviviridae* [6]. *Flavivirus* includes >70 viruses, which are potent human pathogens [7]. These viruses are central etiologic agents of human disease, causing clinical disease ranging from fever to severe manifestations, such as encephalitis and hemorrhagic fever [8]. They are positive-stranded RNA viruses containing a genome of 10–11 kb. Genome is divided into three structural genes—the capsid (C), membrane (M) and envelope (E)—and seven non-structural (NS) genes—NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 [9]. Growing cases of dengue virus (DV) and Japanese encephalitis virus (JEV) in Asia, recurrent outbreaks of yellow fever virus (YFV) in Africa and South America and the continuing spread of West Nile virus (WNV) all through the Americas show the geographical burden of *Flavivirus* diseases. These particular viral diseases are a significant burden on global economies and public health, including the recent Zika virus (ZIKV) and DV diseases, which caused emergency situation across the globe. Accurate diagnosis and differentiation of the infecting virus is important, especially in areas where many *Flaviviruses* are circulating [8].

3. Present flavivirus diagnostic test

Flavivirus diagnosis is often unclear the reason is that the laboratory diagnosis of these viral infections is based on isolation of the virus, detection of viral RNA using reverse transcription polymerase chain reaction (RT-PCR), plaque reduction neutralization test (PRNT), haemagglutination and haemagglutination inhibition assays (HI), antibody detection, immunofluorescence assays (IFA) and enzyme immunoassays (EIA), high-throughput, rapid microneutralisation assays, lateral flow assay (LFA), microsphere immunoassay (MIA), biosensors and microfluidic systems and autologous red blood cell agglutination assays [10]. Most of these assays results may not declare the specific *Flavivirus* infection, since most of them have cross-reactivity or the other issues.

4. Disadvantages of the available tests

The disadvantages of the present available tests include—For PRNT: labour intensive, require skilled personnel, a minimum of 5 days to perform and the handling of live virus, which requires a Biosafety level 3 (BSL-3) facilities. For HI: different pH buffers are required for each different antigen. A constant supply of fresh avian red blood cells is also necessary and there is a high level of cross-reactivity among the *Flaviviruses* [11]. For IFA: requirement for a fluorescent microscope to evaluate the results and cross-reactivity. For enzyme-linked immunosorbent assay (ELISA): unable to differentiate infections caused by different subtypes of particular *Flavivirus*. Accurate diagnosis and differentiation of the infecting virus is impor-

tant, especially in areas where many *Flaviviruses* are circulating. For LFA: sample number should be small. For MIA: requires specialized equipment [12].

5. Need for rapid diagnosis

One of the major challenges during any outbreak is early diagnosis of infection, which is difficult because the early symptoms are non-specific toward particular infection and often are seen in patients with more common diseases [13]. During outbreaks, rapid detection of pathogens in individual cases is crucial in achieving the best clinical management, public health surveillance and control outcomes. With the addition of fields such as chemistry, immunology, molecular biology, biochemistry, nucleic acid amplification and engineering, it is now possible to determine the specific aetiology of a patient's infectious disease in the hospital, clinic, office, remote village or even a patient's home. With automation and highly multiplexed assays, individual pathogens can be readily identified in a wide variety of specimen types, including blood, urine, tissue, mucosal swabs, cerebrospinal fluid, respiratory secretions and stool samples. But one of the major limitations of all present available gold standard techniques is that they require time, which is not possible in case of deadly infectious diseases [5]. Therefore, rapid, accurate and accessible detection of infections have to be done. This has an important role in addressing not only mortality but also the spread of the disease into the newer areas. Rapid diagnostic tests (RDTs) offer the potential to provide accurate diagnosis to all at risk populations those unable to access services such as microscopic and other observation of the specimen. RDTs offer a useful alternative to microscopy.

5.1. Advantages of RDTs

- Personal exposure to patient sample is less.
- Maintaining a specialized laboratory with adequately skilled scientists, technicians and supportive personnel is not required.
- Very less use of high costly instruments and facilities.
- Resources and reagents requirement is limited.
- Discarding of the RDTs equipment is easy.

5.2. Limitations of RDTs

- Environmental conditions such as temperature and humidity can affect the performance of RDTs.
- Packaging, transport and storage are important for proper working of RDTs.

5.3. The ideal RDTs

The properties of RDTs kits play an enormous role in determining their utility in the diagnosis of infectious diseases. The prerequisites of the ideal RDTs include the following:

- Single step processing of the sample.
- Rapid and accurate results.
- RDTs should be cost-effective and sustainable in the long term.
- They have to replace with standard identification test.
- High sensitivity and specificity.
- Reproducible results.
- Purity and volume of assay sample.

6. Serum components as rapid diagnostic markers during flavivirus infections

The short vireamic period in infected hosts means that serological assays are often the diagnostic method of choice. Serum consists of substances that are produced mainly by the body in response to stress, diseases, pathogens, etc. In case of infectious diseases, a serological blood test is performed to detect and measure the levels of antibodies or other serum substances as a result of exposure to a particular microorganism [14]. Studies on serum proteome, cytokines and inflammatory markers during specific *Flavivirus* infection may give us clue for the development of rapid diagnosis. During *Flavivirus* infections since most of the cases are

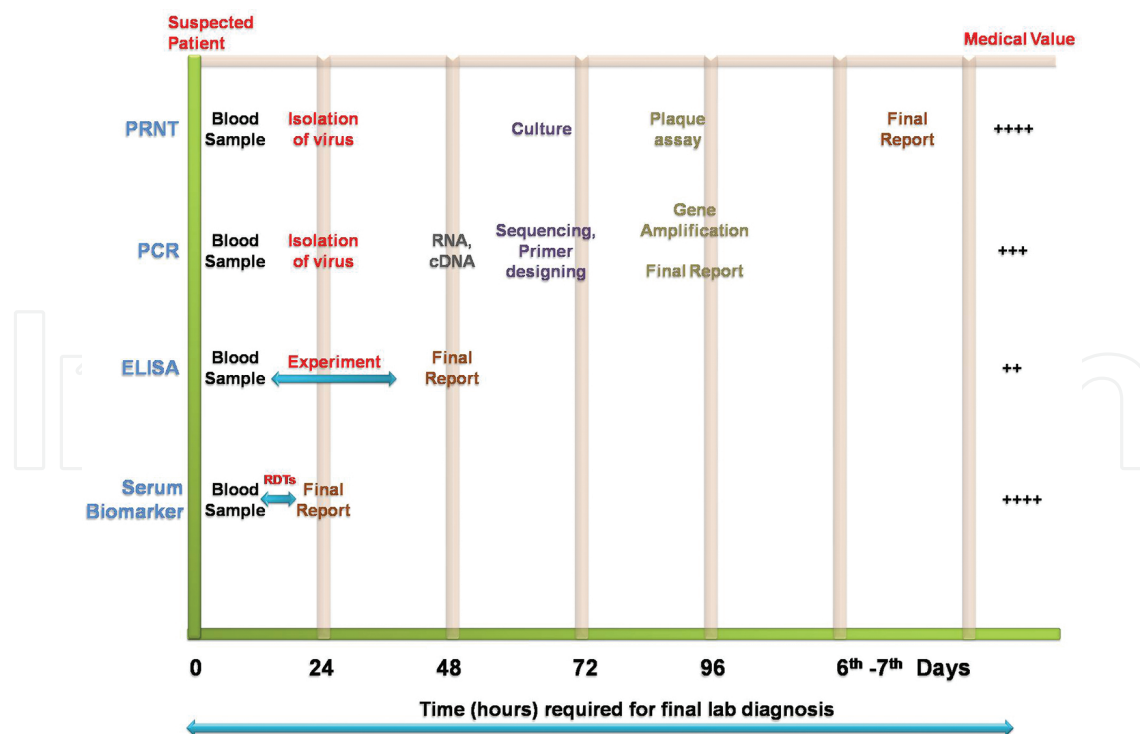


Figure 1. The time taken by the various diagnostic techniques for *Flavivirus* detection and their medical value. Compared to all other available techniques rapid diagnosis using serum offers the best approach to detect the specific *Flavivirus* infection.

asymptomatic during the early phase of the exposure, if the physician has to rely on techniques like PRNT, which is a time consuming, then patient may die, as few patients have short incubation period (**Figure 1**). Serum biomarker analysis for the particular infection and immediate treatment can help the patient to recover soon. The present most precise method for detecting *Flavivirus* infection is PRNT. Even though this method has great insight into this particular virus biology and pathogenesis, it has quite a few boundaries as discussed above. The main weakness that limits the clinical relevance of serological methods (if one is looking for IgG/IgM) is the broad antigenic cross-reactivity that exists between all *Flaviviruses*. The quite specific viral envelope (E) protein neutralizing antibody response may contribute to the false positive results since the amino acid sequences are more conserved. Hence looking for serum biomarkers (other than IgM, IgG) for particular infection can help in rapid diagnostics, least expensive and easy to perform. Here we list out few biomarkers (**Figure 2**) that are available till date for the detection of the *Flavivirus* infections.

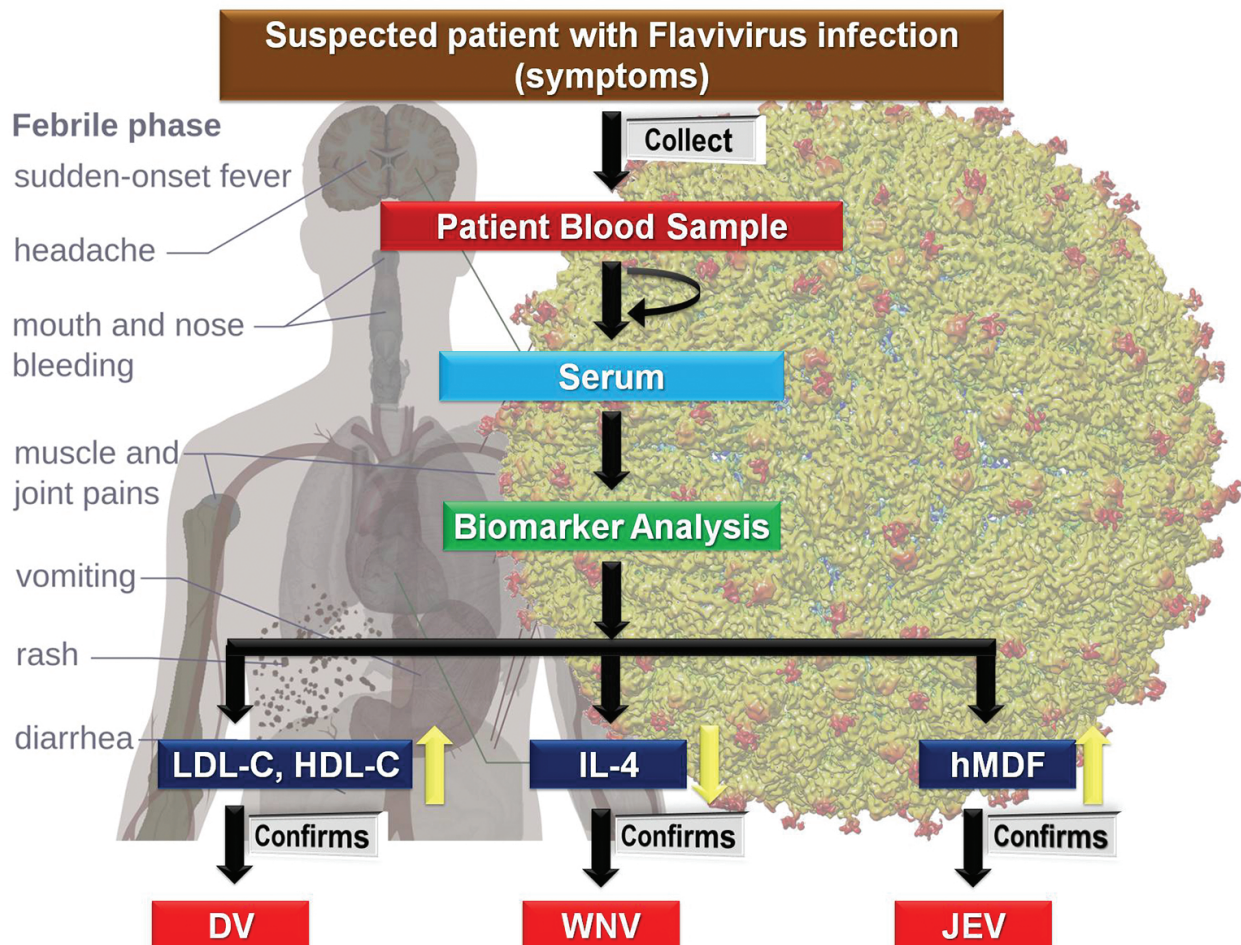


Figure 2. The rapid diagnosis procedure: When an individual with suspected *Flavivirus* infection approaches clinician. The clinician has to go for specific diagnosis based on the symptoms and travel history of the patient. Here are the precise serum biomarkers that are upregulated/downregulated during definite infection.

7. Cholesterol as prognostic biomarker during DV infection

Dengue is a systemic viral infection transmitted between humans by *Aedes* mosquitoes. DV is a *Flavivirus* of global importance, with about four billion people across 128 countries at possibility of infection, and 96 million clinically apparent cases predictable annually [15–17]. The clinical presentation of acute dengue infection is non-specific. The laboratory confirmation of dengue infection relies on isolation of the virus in cell culture, the identification of viral nucleic acid or antigens or the detection of virus specific antibodies [18]. The recent report suggests that the total serum cholesterol, low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C) levels can be used as biomarker during dengue infection since these levels were considerably lower in dengue-positive patients compared to dengue-negative patients. The LDL-C levels showed larger decrease and thus may contribute to the reduction in total cholesterol. Liver damage caused by DV infection may possibly contribute to the lower cholesterol levels. It was also observed that total LDL-C and HDL-C levels were lower in severe compared to mild dengue. In conclusion, lower total serum cholesterol and LDL-C levels at presentation were associated with subsequent development of dengue hemorrhagic fever. In addition, the study also indicates that cholesterol level at presentation may serve as a probable forecaster of severe dengue [19]. Cholesterol and other routine laboratory markers should be explored as a lower cost and more sustainable move toward the development of biomarker that can serve as prognostic marker for dengue viral infection. Another study reported that serum angiopoietin-2 and soluble vascular endothelial growth factor receptor-2 (VEGFR-2) showed a strong correlation with the occurrence of plasma leakage in DV infected patients. It was also suggested they could serve as substitute markers for plasma leakage in patients with acute DV infection [20]. More work need to be done on these markers to confirm that these can be used in rapid diagnosis of dengue infection. There is an urgent need for the development of diagnostic and prognostic tools to identify dengue cases and to provide appropriate supportive care.

8. Serum cytokine profile as biomarker during WNV infection

WNV is a neurotropic human pathogen that is the causative agent of West Nile fever and encephalitis. From 1999 to 2012, 37,088 cases were reported to the US-Centers for Diseases Control, including 1549 fatalities [21]. About one in five people who are infected will develop a fever with other symptoms. Patients typically develop symptoms between 3 and 14 days after they are bitten by the infected mosquito. Centers for Disease Control and Prevention as of September 15, 2015, a total of 46 states in USA have reported WNV infections in people, birds or mosquitoes. Overall, 708 cases of WNV disease in people have been reported. The cumulative incidence of WNV infection may include as many as three million people [22]. The study on serum cytokines found that subjects with a history of severe infection had significantly lower levels of the serum IL-4, whereas asymptomatic subjects had elevated baseline levels of the IL-4 [23]. The study on serum protein profile of healthy and virus infected

individual may give us clue on biomarker discovery and to develop serum based diagnosis of the WNV infection.

9. Diagnosis of St. Louis encephalitis virus

St. Louis encephalitis virus (SLEV) was the leading cause of epidemic *Flaviviral* encephalitis in the United States prior to the introduction of WNV in late 1999 [24, 25]. Preliminary diagnosis is often based on the patient's clinical features, places and dates of travel, activities and epidemiologic history of the location where infection occurred. Majority of SLEV infections remain undiagnosed since the symptoms are like normal flu illness [26]. A standardized IgM antibody-capture enzyme-linked immunosorbent assay protocol is the only available diagnosis for SLEV infection [27]. This assay serves as a priceless tool for the presumptive diagnosis of acute SLEV infections and facilitates the processing of many serum samples. But, the major drawback of this assay is that during the antigen preparation from virus-infected suckling mouse brain the personnel may expose to infectious agents and hazardous chemicals. In addition, the procedure for this antigen preparation is time consuming, costly and tedious [28]. Research need to emphasize on development of rapid diagnosis kit that aids on identification of SLEV at much earlier stage.

10. Serum protein profile as biomarker during JEV infection

According to WHO, global health observatory data repository, there were 4707 reported number of JEV infection cases in 2014 [29]. Japanese encephalitis (JE) is an acute viral zoonotic infection of the central nervous system (CNS), which produces meningoencephalitis. It poses a serious public health problem with an increasing frequency of epidemics and outbreaks in many parts of the Indian subcontinent and Southeast Asian countries [30, 31]. The preliminary diagnosis of JEV is often based on the patient's clinical features, places and dates of travel. The present laboratory diagnosis of JE is generally accomplished by testing of serum or cerebrospinal fluid (CSF) to detect virus-specific IgM antibodies. JEV IgM antibodies are usually detectable 3–8 days after onset of illness and persist for 30–90 days. But the main weakness that limits the clinical relevance of these methods is the broad antigenic cross-reactivity that exists between all *Flaviviruses* and this may contribute to the false positive results. Serum protein profiles other than antibodies work as potential biomarkers for knowing disease status. The analysis of serum proteins of patients and healthy individual by surface-enhanced laser desorption/ionization–time of flight–mass spectrometry (SELDI-TOF-MS) in combination with the ProteoMiner technology that accurately displays low-abundance proteins responsible for virus infection could help physicians for rapid diagnosis of JEV infection [32]. One of the serum factors that could serve as potential biomarker is human macrophage-derived factor (hMDF) that specifically secreted during JEV infection. The levels of hMDF in blood will rise to its highest level by second week of infection [33, 34]. Hence, the detection of hMDF in blood could be a useful prognostic marker of the JEV infection.

11. Conclusion

The burden of *Flaviviral* infection is expected to continue to increase in the future due to climate change, globalization, travel, trade, urbanization, socioeconomics, viral evolution and other factors. The short incubation time for most of the *Flaviviruses* infections and no specific diagnosis make these an important public health problem globally. RDTs methods may be implemented as adjuncts to the epidemiologic investigation of *Flavivirus* disease outbreaks. As sensitivities, specificities, positive predictive value and negative predictive value of RDTs continue to improve and become more widely appreciated through production of less expensive and more user-friendly platforms, it will become necessary to formulate responsible guidelines for the appropriate and optimal use in clinical practice in clarifying infectious disease diagnoses and improving patient outcomes. There is a critical call for discovery of serum biomarkers that are specifically present or up-regulated during particular *Flavivirus* infection. Recently, there were many reports on micro-RNA (miRNA), which gets upregulated or downregulated during these particular infections but relying on miRNA may not be the right practice since they are regulated by several factors and it involves molecular biology work and equipment. The advantage of serum analysis is that, it is least expensive, easy to perform and biosafety practices are not required. Growing cases of *Flavivirus* infections globally says that there is a critical need for the development of diagnostic and prognostic tools to identify specific cases and to provide appropriate supportive care and with anticipation that one day there will be a specific diagnosis and therapeutics for all *Flavivirus* infection.

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