

## The need of an hour for saliva diagnostic research in detection of Ebola viral disease

Dear Editor,

Ebola viral disease is caused by *Ebola virus*. Latest outbreak of this disease in African countries and the fatality rate ranging from 51% to 73% as of June 2014 has caught the attention of the whole world.<sup>[1]</sup> Ebola is a deadly viral hemorrhagic febrile disease that can cause death in 2-5 days.<sup>[2]</sup> Prompt diagnosis is the key for taking steps of prevention. The cultural belief of people in African countries has hampered the collection of blood for diagnostic purpose and thereby led to late diagnosis and transmission of disease.<sup>[3]</sup> Hence, other noninvasive modes of sample collection have been explored.

Saliva has several distinct advantages over other body fluids for diagnosis and prognosis. Oral fluid sampling is safer than blood sampling, eliminates the risk of disease transmission associated with needlestick injuries, is easier, can be collected by patients themselves, is noninvasive, improves patient compliance, and is more economical. Furthermore, saliva does not clot like blood. Oral fluid consists of saliva and crevicular fluid. Whole saliva is widely used for various diagnostic purposes.<sup>[4]</sup> The virus has been isolated in saliva that supports the concept that there is a scope for the salivary antigen or nucleic acid diagnostic test that could be developed for this virus.<sup>[3]</sup> Oral mucosal transudate is rich in antibodies and contains surface pathogens. It is obtained by swabbing the buccal mucosa and tongue. Large number of viruses can be detected in oral samples using an antigen, an antibody or nucleic acid targets.<sup>[3,4]</sup>

Formenty *et al.* in their study obtained serum and oral fluid specimens from patients with suspected Ebola. Specimens were analyzed for immunoglobulin G antibodies by enzyme-linked immunosorbent assay (ELISA) and for Ebola virus by antigen detection ELISA and reverse-transcriptase polymerase chain reaction (RT-PCR). Though the results failed to detect antibodies against Ebola in the oral fluid specimens, RT-PCR results were positive for all oral fluid specimens. Their study demonstrated the usefulness of oral fluid samples for the investigation of Ebola outbreaks.<sup>[3]</sup>

Bausch *et al.*, in their study reported that saliva of half of the patients were tested positive by virus culture and/RT-PCR. The shedding of EBOV in saliva matched exactly to the period of viremia, with the last positive saliva specimen noted at day 8 after disease onset. The study reported higher mortality among patients with RT-PCR-positive saliva, which is an indicator of a poor prognosis.<sup>[5]</sup> PCR-based assays can detect a large array of pathogens in saliva with no interference from PCR inhibitors.<sup>[2]</sup>

Research should be extensively done to improve the detection of antibodies and antigen in oral fluid specimens. Nucleic acid amplification technologies should be improvised to be highly sensitive to oral samples. Adequate salivary samples should be collected by accurately following the manufacturer's instructions. Also, the samples should be stored properly and transported to the laboratory promptly in good conditions. Care should be taken to avoid technical problems in laboratories. Research should also be done on the aspect of possible virus inactivation by salivary enzymes.

Detection of Ebola virus by RT-PCR in oral fluid specimens is sufficiently reliable as a diagnostic tool, especially in the investigation of the outbreak of Ebola. Oral fluid samples for diagnosis should ideally be collected during the acute phase of illness, within the first 10 days of the disease. However, wide ranges of biological investigations are not possible with oral fluid samples; thus, blood samples are still necessary to investigate the biological status and immune response of the patient and to optimize treatment plan. Laboratory methods should be improved to detect suspected cases early and to design more-sensitive and early screening of these diseases, to permit more-rapid responses to disease and implementation of disease-control measures. Thus, the use of oral fluid samples could make earlier detection of outbreaks much easier.

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Letter to Editor

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