Optimization of a Ribonucleic Acid (RNA) Extraction Protocol for Viruses in Clinical Samples for Disease Diagnosis

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Ribonucleic acid (RNA) is a polymeric molecule. It is implicated in coding and gene expression. Some medically important organisms such as viruses have only RNAs as their inherent material. To detect the viral diseases using molecular biological methods, it needs extraction of RNA from body fluids. There are several methods of RNA extraction, which require costly reagents and kits. Hence, the objective of this study was to optimize a low cost, in-house protocol for RNA extraction of viruses in clinical samples in order to facilitate disease diagnosis. Clinically confirmed blood samples, which were positive for Dengue Virus by NS1 antigen test, were taken for optimization of the two protocols. Two different RNA extraction protocols were used for the study to identify the most appropriate and reliable method with high efficiency. Trizol reagent, which was prepared in house was used in both protocols. Extracted RNA from both the protocols were quantified at 260 nm using a spectrophotometer. The RNA amount quantified from the spectrophotometer showed a result of 64 and 72 ng/ul from first and second protocols, respectively. In the first protocol, all the procedures were undertaken at room temperature (27-35 ℃) but generally RNA is not stable at the room temperature. Therefore, RNA might have degraded due to lack of optimum conditions during the incubation, centrifugation and storage periods. In addition, if the RNA pellets were air dried completely, it becomes insoluble in RNase free water. Therefore, extracted RNA might not have been re-suspended completely in the solution. Those identified drawbacks were adjusted in the second protocol. Further, incubation temperature and time period (4 ℃ and 30 minutes) and centrifugation time (15 minutes), were modified to achieve stabilization, complete precipitation of RNA molecules and to prevent degradation by RNases. According to the above discussed facts, this study reveals that the second protocol is more suitable for RNA extraction ofviruses in clinical samples.

Keywords: Ribonucleic Acid (RNA), Virus, Extraction