ONE-STEP REVERSE TRANSCRIPTION QUANTITATIVE PCR ASSAY FOR SIMULTANEOUS DETECTION OF CHIKUNGUNYA, DENGUE AND ZIKA VIRUS BLOOD SAMPLE SPOTTED ON FILTER PAPER

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Abstract. A standardized method using multiplex SYBR Green RT quantitative PCR for direct rapid simultaneous detection of Dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV) viruses in dried blood spotted on filter paper was developed for potential field application. A standard curve was constructed using virus-specific primer pairs and same thermocycling conditions of ten-fold serial dilutions of each stock virus solution in double distilled water or whole blood spotted on filter paper, which were allowed to dry and RNA directly extracted for quantification. Samples prepared immediately and after a 2-month-storage at ambient temperature showed limit of detection (LOD) of DENV, CHIKV and ZIKV in blood following immediate application on Whatman 903 filter paper of 16 PFU/ml for all three viruses and, after storage, 16, 160 and 160 PFU/ml, respectively, while on Whatman 3MM filter paper LOD was 16, 160 and 1,600 and 160, 1,600 and 16,000 PFU/ml, respectively. In conclusion, virus-spiked blood sample applied onto Whatman 903 paper was suitable for direct PCR quantification of DENV, CHIKV and ZIKV, with no change in detection sensitivity of DENV after a 2-month storage but a 10-fold decrease for the other two viruses. This method should prove useful for virus detection of field blood samples in regions where there are co-circulation of DENV, CHIKV and ZIKV.

Keywords: chikungunya virus, dengue virus, zika virus, blood sample, detection, filter paper, RT-quantitative PCR

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