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OPTIMIZATION OF TOTAL RNA EXTRACTION FROM HUMAN URINARY SEDIMENT

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Urine is the best choice to identify biomarkers for metabolic and renal disorders because it is readily available, and samples can be obtained with less harm to patients. However, RNA isolation from voided urine is challenging due to the presence of RNases and cell scarcity. The purpose of this study is to optimize a protocol for RNA extraction from urine samples to be used in gene expression studies. A total of twenty urine samples collected from healthy controls (HC) (n = 11; 49 ± 5 years) and chronic kidney disease (CKD) patients (n = 9; 62 ± 3 years) were centrifuged at 3000g for 30 minutes at 4 ^{o}C. Then, 500 µL of the lysis buffer was added to the pellet, vortexed and kept on ice for 5 minutes. Next, 100 µL of sodium acetate (pH=4.0) and 500 µL of water saturated phenol were added and mixed well. After that, 200 µL of Chloroform: Isoamyl alcohol (49:1) was added, vortexed and centrifuged. An equal volume of cold isopropanol was added to the aqueous phase and incubated at -20 ^{o}C for 1 hour to precipitate RNA. Next, the pellet was washed with 75% ethanol, airdried, and resuspended with 12 µL nuclease free water. Finally, the RNA was quantified and reverse transcribed into cDNA to be used in RT-qPCR. Mean urine volume was 82.5 ± 41.94 mL. Serum creatinine and estimated glomerular filtration rate of CKD patients were 3.04 ± 0.23 mg dL^{-1} and 19.22 ± 4.84 mL/min/1.73 m^2, respectively. Total yield of RNA from CKD and HC samples were 873 ± 523 ng and 735 ± 291 ng, respectively, and a statistically significant difference was not observed between the two study groups ($p > 0.05$). The β2 microglobulin gene could be successfully amplified using samples even with a low cDNA concentration (0.625 ng). This modified phenol-chloroform based urinary RNA isolation method is less expensive, does not require RNA clean-up kits and provides a higher yield of RNA with less inhibition which is sufficient for down-stream applications compared to column-based techniques.

Keywords: Chronic kidney disease, RNA isolation, Urinary sediment.