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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 non-invasively from patients. However, RNA isolation from voided urine is challenging due to the presence of RNases and cell scarcity. This study aims to optimize a protocol for RNA extraction from urine samples in gene expression studies. Twenty urine samples were collected from healthy controls (HC) (n = 11; 49 ± 5 years) and chronic kidney disease (CKD) patients (n = 9; 62 ± 3 years) and were centrifuged at 3,000 g for 30 min at 4 °C. Then, 500 µL of the lysis buffer was added to the pellet, vortexed and kept on ice for 5 min. 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 °C for 1 h to precipitate RNA. The pellet was washed with 75% ethanol, air dried, 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 ± 41.9 mL. Serum creatinine and estimated glomerular filtration rate of CKD patients were 3.0 ± 0.2 mg dL^{-1} and 19.2 ± 4.8 mL min^{-1} 1.73 m^{-2}, respectively. The 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 downstream applications than column-based techniques.

Keywords: Chronic kidney disease, RNA isolation, Urinary sediment