EXPLORING URINARY BIOMARKERS IN NMIBC: ASSESSING SAMPLE QUALITY WINDOW AND THE FEASIBILITY OF IMMUNE PROFILING IN URINE SAMPLES

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Background Assessing urinary biomarkers is crucial for understanding immune responses and therapeutic efficacy in non-muscle invasive bladder cancer (NMIBC) and other urological pathologies. However, challenges arise from low urine cellularity, the need for biomarker normalization, and uncertainties regarding biospecimen stability during sample transit and laboratory processing. This study was designed to address the stability of urine leukocyte composition, establish convenient logistics, and explore protein composition analysis feasibility while addressing normalization factors.

Methods Stability of leukocytes from four healthy donors (HD) spiked into urine supernatant was analyzed for up to 72 hours at 4°C. Urine samples from five NMIBC patients undergoing BCG treatment were divided into two portions, followed by either immediate processing or overnight refrigeration. Urine samples from eight untreated NMIBC patients and age-matched HD were shipped overnight at 4°C for immediate processing on arrival. Urinary cell content and soluble factors were analyzed by multiparameter flow cytometry and Luminex® respectively. Protein concentrations were normalized to total urine protein or creatinine.

Results We demonstrate stability of spiked peripheral blood leukocytes in urine matrix up to 72 hours at 4°C without viability loss or changes in leukocyte subset distribution. Overnight refrigeration of urine samples from BCG-treated patients did not affect viability or total counts of CD45+ cells, granulocytes, and monocytes. Slight decrease in T lymphocyte numbers was observed, but most samples could still be analyzed, with only 2 out of 16 samples falling below the limit of detection in the CD3, CD8, or CD4 gates. Samples from untreated NMIBC patients and age-matched HD could be analyzed for granulocytes, g-MDSCs, monocytes, m-MDSCs, T cells (CD8 and Tcon subsets) and NK cells, as well as for functional marker expression (PD-1, PD-L1, and HLA-DR).

Finally, multiple proteins were successfully quantified in urine supernatant of all patients and HD, and protein concentrations could be normalized to total urine protein or creatinine.

Conclusions Our results demonstrate that overnight urine shipment at 4°C followed by flow cytometry upon delivery is feasible without loss of urine leukocyte numbers and viability. Furthermore, proteomic analysis of overnight refrigerated samples is feasible. These data validate urine as a source of key biomarker data and establish sample quality window for successful analysis of immunological parameters in patients’ samples.

Ethics Approval The studies were approved by the Health Research Authority, London – Stanmore Research Ethics Committee (19/LO/0179; 257743) and the Ethics Committee of the Canton de Vaud in Switzerland (#2019–00564).

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