# New nanoparticle aided glycovariant biomarker tools to detect extracellular vesicles as a liquid biopsy for early diagnosis of bladder cancer

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## Introduction

Bladder cancer (BlCa) diagnosis requires sensitive and non-invasive methods. Our study aims to develop a nanoparticle-based immunoassay (TRFIA) utilizing biotinylated antibodies as capture agents and *Ulex Europeaus Agglutinin* (UEA) as a tracer. Focusing on potential markers including EpCAM, CAM1, and ITGA-3, we optimized conditions and observed p-values of .03, .04, and .05, respectively. These findings underscore the critical need for exploring UEA lectin screening with various capture agents to enhance BlCa detection in a non-invasive manner.

## Materials and Methods

Our methodology involved the extensive optimization of conditions for the TRFIA assay. Biotinylated antibodies were strategically selected as capture agents, targeting EpCAM, CAM1, and ITGA-3, while UEA lectin served as the tracer molecule. Glycan on BlCa and benign samples are detected by UEA lectin which was conjugated on 97nm polystyrene Europium Nanoparticles ($Eu^{+3}$-NPs), each nanoparticle contains 30000 fluorescent $Eu^{3+}$ chelates**.** The screening process involved varying combinations of capture agents and UEA lectin to maximize sensitivity and specificity for BlCa detection.

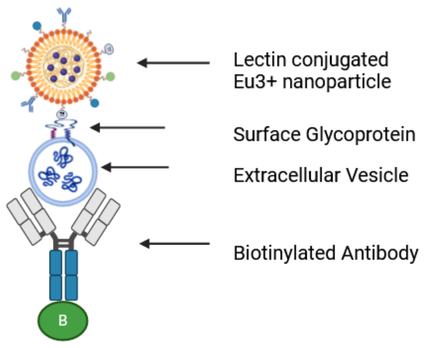


**Figure 1. A schematic representation of mechanisms of TRFIA assay.** Biotinylated antibody is immobilized in the streptavidin-coated plate followed by the recognition of antigen. Glycan on EVs are detected by lectin coated on NPs. *Figure drawn in Biorender 2024.*

## Results

Our study demonstrates the substantial impact of optimizations on assay performance, leading to a marked enhancement in sensitivity and specificity. Furthermore, through the screening of UEA lectin with various capture agents, we tried to develop an assay showcasing remarkable accuracy in discriminating bladder cancer samples from benign ones, representing a significant improvement over current methodologies.

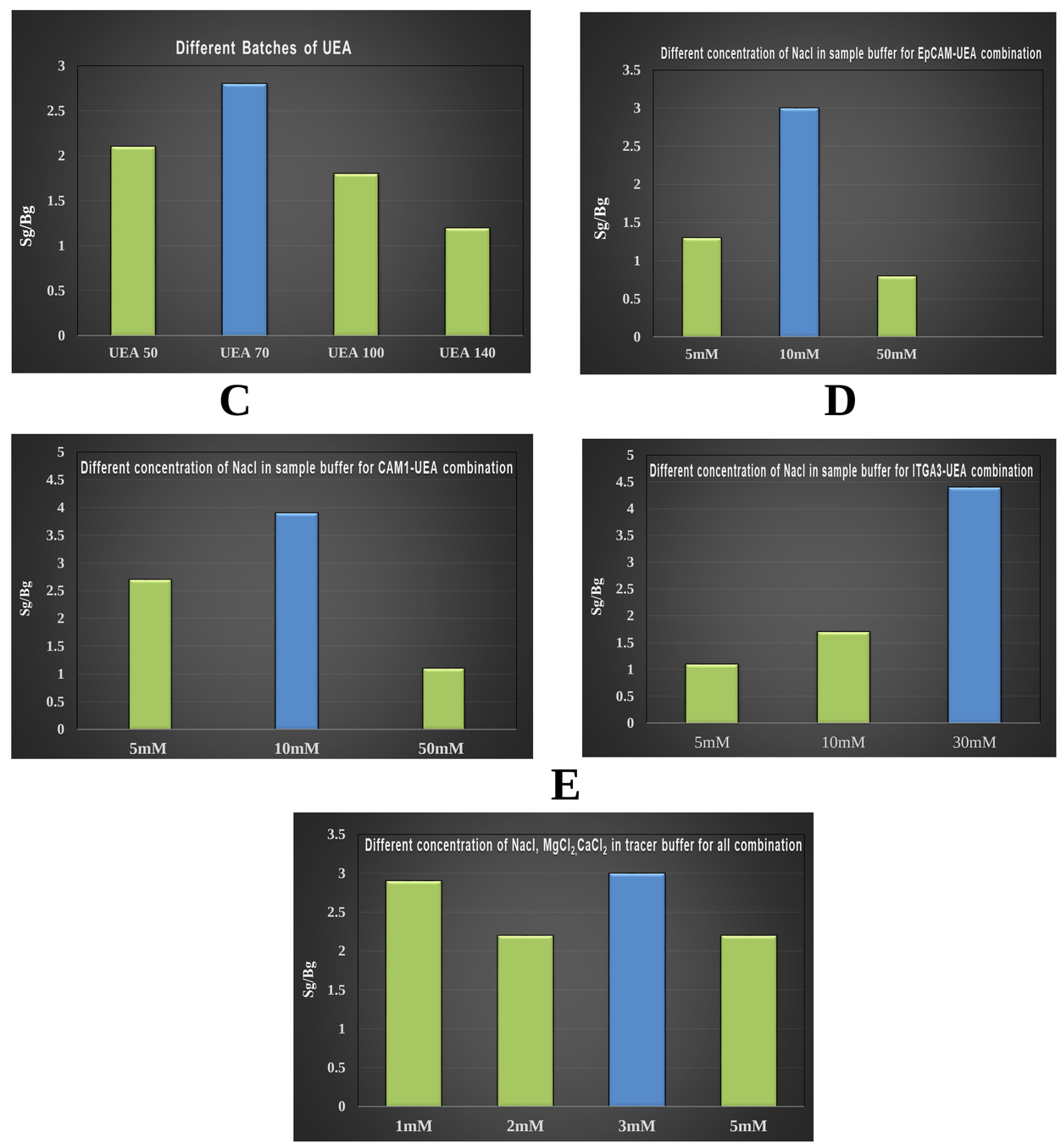


**Figure 2. Optimization of assay using different batch of tracers and salt concentration. (A)** For best tracer selection, we used four different batches of UEA tracers, identifying UEA70 as the most effective batch among the combinations. **(B,C,D)** To determine the optimal salt concentration in the sample buffer, we conducted assays using varying NaCl concentrations in EpCAM-UEA, CAM1-UEA, and ITGA3-UEA assays. Results indicated that 10mM NaCl yielded the highest signal-to-background ratio for EpCAM-UEA and CAM1-UEA, while 30mM NaCl was optimal for ITGA3-UEA. **(D)** Similarly, for getting optimal salt concertation in tracer buffer, we used a combination of NaCl, $MgCl_2$, and $CaCl_2$ salt concentrations. Among these, a concentration of 3mM demonstrated best performance in terms of signal-to-background ratio.

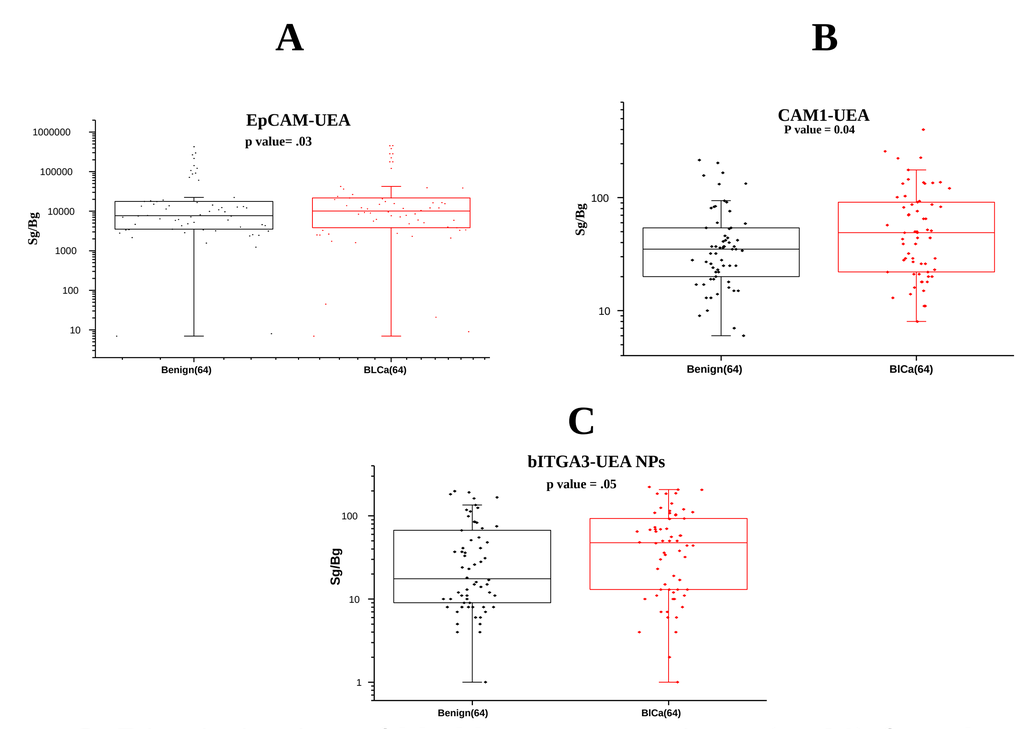


**Figure 3.** Discrimination of bladder cancer patients (n=64) from benign controls (n=64) using nanoparticles coated UEA as tracer and biotinylated EpCAM (A), CAM1 (B) and ITGA-3 (C) as immunocapture. The p-values were calculated with Mann-Whitney U test from the signal-to-background (S/B) values.

## Conclusion

The development and validation of this highly sensitive nanoparticle-based TRFIA hold immense promise for improving the accuracy and efficiency of BlCa detection. Further research and clinical validation are required to use these findings into clinical practice, ultimately advancing early detection and patient outcomes in BlCa management.