TOWARDS A NO-WASH ELECTROCHEMICAL IMMUNOASSAY FOR 25-OH VITAMIN $D_3$. **Aaliya Pathan**, Darius Rackus, Department of Chemistry and Biology, Toronto Metropolitan University, 350 Victoria St., Toronto, ON M5B 2K3, Canada,; Institute for Biomedical Engineering, Science, and Technology (iBEST), St. Michael's Hospital, Toronto, ON M5B 1W8, Canada; Keenan Research Centre for Biomedical Science at St. Michael's Hospital, Toronto, ON M5B 1W8, Canada; Graduate Program in Molecular Science, Toronto Metropolitan University, 350 Victoria St., Toronto, ON M5B 2K3, Canada.

Vitamin D3 deficiency is highly prevalent, making it one of the biomarkers for several chronic diseases. As a result, there has been a demand for portable testing platforms to enhance disease management and monitoring accessibility. Currently, determination of vitamin D levels relies on laboratory-based immunoassays which face challenges such as interference through non-specific adsorption and the need for washing to remove unbound labels, making it complex and time-consuming. To address this challenge, we report a wash-less electrochemical competitive immunoassay to measure free 25-OH Vitamin D3 (25-OHD). The assay is based on passivation of the electrode surface resulting in a decrease in electron transfer for the reduction of $Ru^{3+/2+}$. Cyclic voltammetry and electron impedance spectroscopy were used to interrogate the electrode surface in response to varying concentrations of 25-OHD. In a one-pot "no-wash" protocol, a limit of detection of 30 ng/mL was observed. The no-wash assay was improved by orienting capture antibodies immobilized on the electrode surface through protein A or by replacing the capture antibody with VDBA14 aptamer. While further reduction of signal-to-noise is needed for a lower LOD, these findings demonstrate the potential for a rapid, simple electrochemical, and quantitative point-of-care test for vitamin D.