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**Presentation Title:** A novel ELISA assay for human kallikrein 5, a candidate breast and ovarian cancer biomarker

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**Author Block:** *Iacovos P. Michael, George M. Yousef, Antoninus Soosaipillai, Linda Grass, Eleftherios P. Diamandis.* Department of Laboratory Medicine and Pathobiology, University of Toronto & Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada, Discipline of Pathology, Health Care Corporation of St. John's, St. John's, NF, Canada, Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada

Human kallikrein 5 (hK5; encoded by the *KLK5* gene) is a member of human kallikreins (hKs); a family of serine proteases. Several members of this family have been shown to be candidate biomarkers for breast, ovarian and prostate cancer, with hK3/PSA (prostate specific antigen) being an established biomarker for prostate cancer diagnosis and monitoring. In previous studies we have shown that at the mRNA level, *KLK5* is a marker of unfavorable prognosis for ovarian and breast cancer. At the protein level, hK5 is a candidate diagnostic marker, since it is elevated in serum of a proportion of breast and ovarian cancer patients.

In this study we aimed to develop a specific enzyme linked immunosorbent assay (ELISA) for hK5, in order to examine hK5 as a serum biomarker for endocrine-related malignancies. Recombinant hK5 was produced in the *Pichia pastoris* expression system and purified by cation exchange chromatography. Purified recombinant hK5 was used for the production of anti-hK5 monoclonal antibodies. Subsequently, a sandwich-type ELISA with monoclonal-monoclonal antibody configuration, coupled with time-resolved fluorometric detection, was developed. The hK5-ELISA has a detection limit of 0.05µg/L and is specific for hK5, with < 0.05% cross-reactivity with the remaining 14 members of this family.

We used this ELISA to measure levels of hK5 in 37 normal tissue extracts, 10 biological fluids (ascites fluid of ovarian cancer patients, follicular fluid, sweat, cerebrospinal fluid, breast milk, saliva, seminal plasma, amniotic fluid, breast cancer cytosolic extracts and urine) and conditioned media from 1 astrocytoma, 9 breast cancer, 1 cervical cancer, 1 colon cancer, 3 neuroblastoma, 2 osteosarcoma, 10 ovarian cancer, 1 pancreatic cancer, 5 prostate cancer and 1 skin keratinocyte cell lines. hK5 was significantly expressed in skin (12 µg/g total protein), breast (1.2 µg/g), salivary glands (0.5 µg/g) and esophagus (0.5 µg/g) and also detected in many brain regions. hK5 was also found in all tested biological fluids with higher amounts in breast milk (19.9 ± 2.4 µg/L). Relatively high levels of hK5 were also found in ascites fluid of ovarian cancer patients (62 ± 149 µg/L). Among the cancer cell lines, high amounts of hK5 were found in supernatants of the breast cancer cell line MDA-MB-468 (237 µg/L), the ovarian cancer cell lines HTB-75 (120 µg/L), HTB-19 (146 µg/L) and HTB-161 (85 µg/L), the prostate cancer cell line PC3(AR6) (250 µg/L), the pancreatic cancer cell line MiaPaCa (65 µg/L) and the skin keratinocyte cell line HaCaT (68 µg/L).

In conclusion we developed a novel ELISA assay specific for hK5 and quantified hK5 in various tissues, biological fluids and conditioned media from cell lines. This assay will be a useful tool for further examination of hK5 as a cancer biomarker.

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