**Accepted for publication in Nature Scientific Reports**

**Simultaneous Analysis of Urinary Total 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol, *N*′-Nitrosonornicotine, and Cotinine by Liquid Chromatography-Tandem Mass-Spectrometry**

**Sampada S. Nikam1, Murari Gurjar1, Hitesh Singhavi2, Anand Patil1, Arjun Singh2, Peter Villalta3, Pankaj Chaturvedi2, Samir S. Khariwala4, Vikram Gota,1,\*   
and Irina Stepanov3,4,5,\***

1 Advanced Centre for Treatment, Research and Education in Cancer (ACTREC)

2 Tata Memorial Centre, Mumbai, India

3 Masonic Cancer Center, University of Minnesota

4 Department of Otolaryngology, Head and Neck Surgery, Medical School, University of Minnesota

5 Division of Environmental Health Sciences, School of Public Health, University of Minnesota

Biomarkers of exposure to harmful tobacco constituents are key tools for identifying individuals at risk and developing interventions and tobacco control measures. However, tobacco biomarker studies are scarce in many parts of the world with high prevalence of tobacco use. Our goal was to establish a robust method for simultaneous analysis of urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), N′-nitrosonornicotine (NNN), and cotinine at the Advanced Centre for Treatment, Research and Education in Cancer (ACTREC) in Mumbai, India. These biomarkers are validated measures of exposure to the carcinogenic tobacco nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and NNN and the addictive alkaloid nicotine, respectively. The established method is characterized by excellent accuracy, linearity, and precision, and was successfully applied to the analysis of 15 smokeless tobacco (SLT) users and 15 non-users of tobacco recruited in Mumbai. This is the first report of establishment of such procedure in a laboratory in India, which offers the first in-country capacity for research on tobacco carcinogenesis in Indian SLT users.