QUALITY CONTROL OF TOTAL NON-ENZYMATIC SEMINAL ANTIOXIDANT CAPACITY BY AN ENHANCED CHEMILUMINESCENCE ASSAY.


The non-enzymatic antioxidants in seminal plasma protect the sperm against oxidative insult. Accurate and reliable assessment of total non-enzymatic antioxidant capacity (TAC) of seminal plasma is essential for both research and clinical purposes. The objectives of this study were to assess the inter-donor (different donor samples measured in the same day), intra-assay (running the same sample in duplicate or triplicate), inter-assay (same sample observed on different days by the same observer), and inter-observer (multiple observers on the same day with the same sample) variability using an enhanced chemiluminescence assay. Semen samples were obtained from normal donors (n=5) and from infertile men (n=5) attending the male infertility clinic. Liquefied semen samples were centrifuged at 250g for 7 minutes and aliquots of seminal plasma were stored at -80°C. TAC was measured with the luminometer set in the kinetic mode. Trolox (6-hydroxyl-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), a water-soluble tocopherol analogue, was used as a standard. Results were expressed as Trolox equivalents. Significantly higher TAC [mean ± standard deviation (SD)] was seen in donors (1324 ± 191) compared to patients (542 ± 184) (P <0.0001). The intra-assay SD was 41 with an intra-assay reliability of 91% (coefficient of variation (CV=5%). The inter-assay SD was 111 with an inter-assay reliability of 92% (CV=13%). The inter-observer SD was 115 with an inter-observer reliability of 89% (CV=13%). Our results demonstrate that enhanced chemiluminescence assay is both accurate and reliable for measurement of TAC levels in seminal plasma.