ASSESSMENT OF LABORATORY VARIABILITY IN THE MEASUREMENT OF TOTAL NON-ENZYMATIC ANTIOXIDANT CAPACITY OF SEMEN USING AN ENHANCED CHEMILUMINESCENCE ASSAY

Ramadan A. Saleh, Hiroshi Kobayashi, Pavithra Ranganathan, Rakesh K. Sharma, David R. Nelson, Ashok Agarwal, The Cleveland Clinic Foundation

Objective:
It has been established that non-enzymatic antioxidants in seminal plasma are as important as enzymatic antioxidants in protecting the sperm against oxidative stress-induced damage. 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 measured 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.

Design:
Quality control study in an Andrology laboratory.

Materials/Methods:
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 for separation of seminal plasma. Aliquots of seminal plasma were stored at -80°C. A standard solution of Trolox (6-hydroxyl-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), a water-soluble tocopherol analogue, was used at three different concentrations (25, 50 and 75 µM) for calibration. TAC was measured in seminal plasma from the donors and the patients with the luminometer set in the kinetic mode. Results were expressed as Trolox equivalents.

Results:
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%).

Conclusions:
Our results demonstrate that enhanced chemiluminescence assay is both accurate and reliable for measurement of TAC levels in seminal plasma. Use of this standardized protocol for measurement of TAC in semen will help assessment of the oxidative stress status of infertile men. In addition, patients with low levels of seminal TAC may be good candidates for antioxidant supplementation.

Supported by:
A grant from the Cleveland Clinic Foundation.