

Journal of Andrology, Vol 22, Issue 4 568-574, Copyright © 2001 by The American Society of Andrology

JOURNAL ARTICLE

Quality control of reactive oxygen species measurement by luminol-dependent chemiluminescence assay

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A total of 28 donor semen samples were used to evaluate the characteristics of laboratory variability in measuring reactive oxygen species (ROS). The objectives of this study were to assess the interassay (same sample observed on different days by the same observers) variability; interdonor, intraobserver (replications of the same sample on the same day) variability; and interobserver (multiple observers on the same day with the same sample) variability of the luminol-dependent chemiluminescence assay and to establish an optimal semen age and sperm concentration. Semen samples were collected from 6 healthy donors for 108 measures of ROS. ROS levels were measured by the assay using luminol as the probe. An additional assessment measured the effect of time (age of the sample) on ROS production in 12 donor samples at 60, 120, 180, and 240 minutes after the specimen was produced. Last, to evaluate the effect of sperm concentration on ROS production, ROS levels were measured in 10 donor sample aliquots with sperm concentrations ranging from 1 to 120 x 10⁶/mL. In the controls, the mean ROS level was 0.218 x 10⁶ counted photons per minute; the interassay variability standard deviation (SD) was 0.077. The interobserver SD was 0.002 for an interobserver reliability of 97.5% (coefficient of variation [CV] = 0.9%). The intraobserver (between replication) SD was 0.001 for an intraobserver reliability of 98.7% (CV = 0.5%). The interassay SD was 0.005 for an interassay reliability of 93.8% (CV = 2.0%). There was no statistically significant interobserver, intraobserver, or interassay variation (P > .80). ROS levels decreased significantly with time; a dramatic decline in ROS production was seen in the specimens that were more than 60 minutes old (P < .001). ROS values decreased by 31% at 120 minutes and 62% at 180 minutes compared with the 60-minute-old specimens. A linear relationship was seen between the ROS levels and sperm concentration in 8 of the 10 samples analyzed (R² = .99). Our results demonstrate that the luminol-dependent chemiluminescence assay for ROS measurement is both accurate and reliable when the sperm concentration is greater than 1 x 10⁵/mL and the samples are analyzed within the first hour after specimen collection.

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