Validation of a Commercially Available Fluorescence-Based Instrument to Evaluate Stallion Spermatozoal Concentration

Kathryn L. Comerford, BS; Charles C. Love, DVM, PhD; Steven P. Brinsko, DVM, MS, PhD; Ann J. Edmond, BS; Jessica A. Waite, BS, MS; Sheila R. Teague, BS, MS; and Dickson D. Varner, DVM, MS

The NucleoCounter® SP-100 instrument has comparable accuracy to the hemocytometer and the flow cytometer at spermatozoal concentrations of 0-800 million/mL and has an advantage for measuring spermatozoal concentration over the previously used photometric systems because it is able to accurately analyze spermatozoa in ejaculates that are contaminated with leukocytes, red blood cells and urine. It is also far less laborious to use than the flow cytometer and the hemacytometer. Authors’ address: Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843; e-mail: katiecomerford@hotmail.com (Comerford). © 2008 AAEP.

Raw stallion ejaculates (n = 120) were analyzed for sperm concentration with a newly developed fluorescence-based sperm counter, and these values were compared with flow cytometric and hemacytometric methods, which are accepted as “gold standards” for measuring sperm concentration. Results were compared statistically by (1) regression analysis (intercept, slope, and coefficient of determination) and (2) testing level of agreement using a modified method of Bland and Altman, whereby the percentage of difference in values between two instruments divided by the mean of the same two values was plotted (y-axis) against the mean value of two instruments (x-axis) to determine a percentage of deviation. Results of regression analysis between NucleoCounter and flow cytometer or hemacytometer were $R^2 = 0.95, 0.92$; slope = 1.14, 1.05; and y-intercept = 8.60, −6.00, respectively. The average percentage deviation of the NucleoCounter compared with the flow cytometer or the hemacytometer in the concentration ranges of 0–200, 200–500, and 500–800 million/mL were 13.5% and 18.2%, 11% and 10%, and 16.2% and 17.3%, respectively. Within-sample repeatability measurements for the NucleoCounter, flow cytometer, and hemacytometer were 3.17%, 2.95%, and 6.69%, respectively. In conclusion, this study indicates that the NucleoCounter is an accurate and precise instrument for the measurement of sperm concentration in raw stallion semen, having acceptable agreement with both the flow cytometer and the hemacytometer over a wide range of sperm concentration and providing good repeatability.

The authors thank Chemometec, Allerød, Denmark, for materials support. Financial support was provided by The Patsy Link Equine Endowment.
Fund, Texas A&M University. The authors have no financial interests with Chemometec or with companies that manufacture or sell competing products.

**Reference and Footnote**


*NucleoCounter SP-100; ChemoMetec, Allerød, DK-3450 Denmark.*