

Appendix IX: Product Performance Data

Abbreviations:

TSC:	Total Sperm Concentration (Count)
MSC:	Motile Sperm Concentration
OD:	Optical Density
SD:	Standard Deviation
CV:	Coefficient of Variation

Performance Data Summary:

The performance of the SQA-Vt is summarized in the text, tables and graphs below. All sperm concentration measurements are expressed as 10^9 sperm cells per milliliter (Billion/ml). Motility is expressed as a percent (%). Unless otherwise noted all testing was performed using turkey semen samples.

Calibration:

Each SQA-Vt is biologically calibrated against two reference devices at Medical Electronic System's laboratory using turkey semen stored in liquid nitrogen.

Table 1. SQA-Vt Dynamic Range

Sample Type	TSC Bil./ml	Motility %	MSC Bil./ml
Fresh turkey semen	0-20	0-100	0-20

Precision and accuracy is based on in-house and field trials using turkey semen samples

Performance claims:

Precision (CVs)

- TSC: 4.0%
- MSC: 5.5%
- Motility: 6.0%

Accuracy (regression coefficients of the dilution and "live/dead" trend line)

- TSC: 0.90
- MSC: 0.90
- Motility: 0.9

Correlation to Manual Method

- Concentration: 0.90
- MSC: 0.65

Notes regarding performance claims:

1. Precision CV claims are higher (this represents overall lower precision) than actual values noted (Table 2).
2. Accuracy regression coefficient claims are less than actual values noted (Fig. 1 - 5).
3. Correlation to Manual Method claims are less than actual correlations noted (Fig. 6 & 7).

Precision and Accuracy

Background: A total of 71 turkey semen samples were analyzed.

Intra and Inter-device coefficients of variation (CV) were run to determine the precision of the system. The following formula is used to calculate CV:

$$CV = SD / MEAN \times 100$$

The lower the CV, the higher the precision of the method (Table 2).

Turkey semen was sequentially diluted and measured on the SQA-Vt to assess accuracy against an "expected" value. Dilution plots with regression coefficients were constructed (Figures 1 & 2).

Precision assessment: Each sample dilution was run in triplicate on the same SQA-Vt. Intra-device CV was calculated from these readings. Inter-device CV was calculated by running the same turkey semen samples on two different SQA-Vt systems. Two trials were conducted.

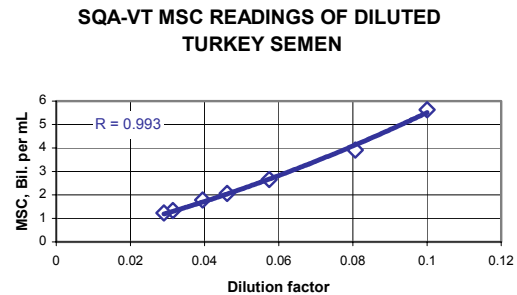
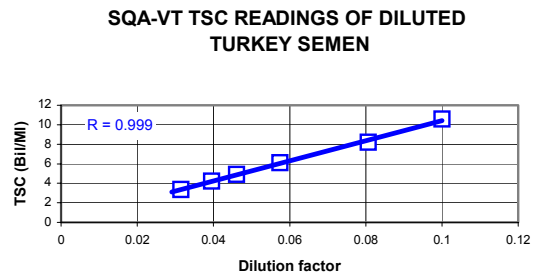
Accuracy assessment: dilution plots. Accuracy was determined by testing different dilutions of fresh turkey semen providing varying TSC and MSC and relatively stable Motility. The TSC and MSC results were plotted vs. dilution factor. The linear trend line was created for TSC values (Fig. 1). Because motility is more variable than concentration, a second-degree polynomial trend line was generated for the MSC data points (Fig. 2). The regression coefficients for TSC and MSC were 0.999 and 0.993 correspondingly.

Accuracy assessment: "live/dead" sperm plots. A "live/dead" study was conducted to assess the accuracy of the SQA-Vt. Pooled fresh turkey semen was divided into two aliquots. The first aliquot was intact ("live") and the second aliquot was treated with liquid nitrogen ("dead"). Samples were prepared with varying proportions of "live-to-dead" cells while maintaining a constant TSC (varying MSC and Motility only). The samples were tested on both the SQA-Vt and under the microscope. Results are plotted in Figures 3, 4 & 5. Linear trend lines were established for motility vs. the "Live/Dead" sperm ratio and vs. the microscope. A second-degree polynomial trend line was generated for the MSC data points.

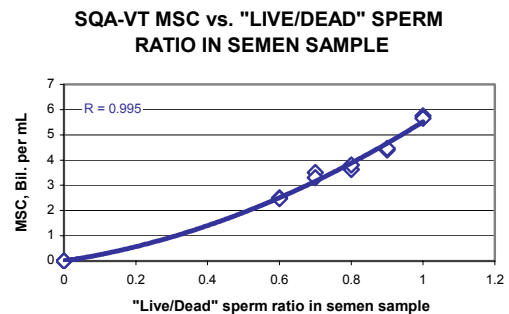
Table 2. Precision, CV (%)

SQA-Vt	TSC	MSC	Motility
Intra-device Variability	3.69	4.99	5.41
Inter-device Variability	1.50	4.55	5.64

Accuracy: Shown in Figures 1 & 2 Dilution plots with regression coefficients R



Accuracy: Figure 3, 4 & 5. "Live/Dead" sperm plots with regression coefficients R



Conclusion:

The SQA-Vt provides precise and accurate results with low coefficients of variation (<6%) and high regression coefficients of dilution and "live/dead" trend lines (>0.95).

Method comparison

A total of 60 turkey semen samples were used to run TSC and MSC on the SQA-Vt and to compare these results to a standard microscopic assessment using a Makler counting chamber and a standard slide and based on the WHO'99 guidelines and MES protocols. Fresh turkey semen samples were diluted with 0.9% NaCl, pH 7.0. Regression plots were generated (Figure 6 & 7). The slope equations and correlation coefficients were reported. The slope coefficient for TSC was 1.0 and MSC 0.9 demonstrating no systematic error. The TSC correlation coefficient was 0.97 and MSC was 0.7.

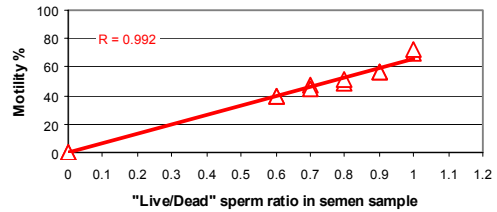
Limitations of method:

Microscopic semen analysis is highly subjective and the accuracy is impacted by statistical counting errors. 95% Confidence interval is equal to +/-1.96 Standard deviations (SD) of the mean value. SD is a square root of the number of spermatozoa counted.

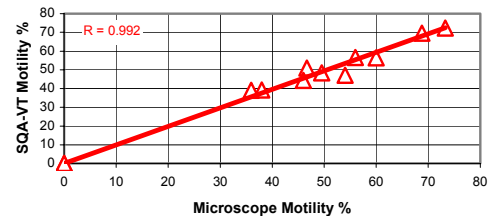
Conclusion:

The SQA-Vt accurately assesses turkey semen compared to manual methods and therefore can be used interchangeably and/or replace manual methodology.

SQA-VT MOTILITY vs. "LIVE/DEAD" SPERM RATIO IN TURKEY SEMEN SAMPLE

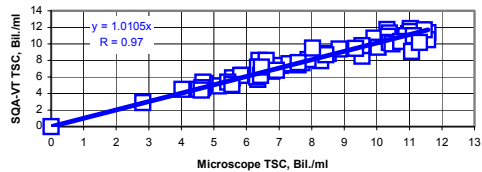


SQA-VT vs. MICROSCOPE MOTILITY IN "LIVE/DEAD" SPERM EXPERIMENT



**Method comparison: Figures 6 & 7
Regression plots with correlation coefficients R**

TSC: SQA-VT vs. MICROSCOPE



MSC: SQA-VT vs. MICROSCOPE

