Appendix X: SQA-Ve System Specifications

Dimensions: 40 x 30 x 15 cm

Weight: 4 kg

AC power supply: 100 to 250 VAC, 50/60 Hz, 10 VA

Measurement Compartment

- **Sources of radiant energy** - two 880 nm LEDs for motility and spectrophotometry channels
- **Detector system** – 2 photo detectors - Motility and Optical Density

Visualization Compartment

- Green LED illumination system
- CCD, 350 TV lines
- Objective lens: Standard, x20
- Signal Output: PAL standard
- Zoom system for smooth magnification transition from x300 to x500
- Focus regulator

Display(s)

- Operational backlight LCD (16 lines x 40 characters)
- Video backlight LCD (8 x 10 cm)

Printer

- Built-in, Dot Matrix
- Non-thermostatic narrow paper with 20 characters per line (Citizen)
- Ribbon cassette (Citizen)

Keypad

- **Operational keys:** ON/OFF, TEST, PRINT, SERVICE, ARCHIVE (disabled), DELETE, ENTER, four cursor buttons, ESC, numeric buttons (0-9)
- **Video control keys**: ZOOM IN/OUT, ILLUMINATION HIGH/LOW, and MONITOR ON/OFF

Front Panel

- Built-in printer
- Visualization compartment
- LCD video display and controls
- Focus knob
- LCD operational display
- Measurement compartment
- Multi-button keypad

Rear/Side Panel

- Power connector with fuse-holder (fuse 250V, 1A)
- Video connector
- RS232 cable outlet
- I-Button port (side panel)

Specimen Testing Supplies

- **Measurement capillary:** Disposable, multi-use plastic, positive displacement testing capillary (purchase from manufacturer).
- **Standard lab slide:** 76 x 25.6 mm, 22 x 22 mm cover-slip.
- **I-Button:** Required to run tests (purchase from manufacturer)

Operating System

- **Control:** Keypad
- **Analysis Time:** 45 seconds

- **Software:** Resides on flash memory and drives all man-machine interface functions, runs algorithms for test measurements and operational screens. System can be upgraded from a PC CD-ROM.
- **Sample Testing Temperature:** 37^{0}C (98.6^{0}F).
- **Motility channel input signal:** Analog, up to 5V.
- **Spectrophotometer channel input signal:** Modulated (1 kHz) analog, up to 5V.

Quality Control

- **Internal:** Electronic Self-Test and Auto-Calibration.

PC Compatibility

Minimum requirements for E-Sperm™ software

- **PC:** 1 GHz processor, Pentium 3
- **RAM:** 256 MB
- **CD ROM drive**
- **Ports:** One serial

Operating system compatibility: Windows XP and VISTA

Operational Temperature and Humidity

- System is operational at 15-40°C.
- *NOTE*: SQA-Ve operates in a wide range of ambient temperatures however the system is calibrated to measure semen samples at 37^{0}C (98.6^{0}F).
- System is fully operational at up to 80% humidity and 31°C.

Maintenance Schedule

- Cleaning daily and after every 50 tests (refer to User Guide – "Cleaning Instructions").

Manufacturer Recommendations

- Operate the SQA-Ve away from devices that may cause electronic noise (cell phones) or other devices causing vibrations such as centrifuges.
- Turn system **OFF** at the rear-panel when not in use for extended period of time.
- Semen is considered a biologically hazardous material and is subject to individual laboratory protocols for handling such materials.

Factory Default Settings:

Date format: DD/MM/YY

Time/Date: Manufacturer's local time/date

Automatically print: YES

Appendix XI: SQA-Ve EQUINE Product Performance Data

Performance Data Summary:

The performance of the SQA-Ve is summarized in the text, tables and graphs below. All values concerning sperm concentration measurements are expressed as 10^6 sperm cells per milliliter (M/ml). Motility values are expressed as a percent (%). Unless otherwise noted, all testing was performed using raw, extended, cooled and frozen equine semen samples. Manufacturers claims are generally lower than actual performance data. Please also note that Sensitivity & Specificity are clinical screening parameters that demonstrate the accuracy of device. Sensitivity demonstrates the ability of the SQA-Ve to correctly detect ABNORMAL cases. Specificity demonstrates the ability of the SQA-Ve to correctly detect NORMAL cases. Sensitivity & Specificity results are based on the cutoffs established by Society of Theriogenology. Each SQA-Ve device is biologically calibrated against two reference systems at Medical Electronic System's laboratory.

Abbreviations:

CONC: Sperm Concentration
CV: Coefficient of Variation
M/ml: Million per milliliter

Table 1. Dynamic Range

Sample Type	Concentration M/ml	% Motility	% Progressive Motility	% Normal Morphology
Raw	0-800	0-100	0-100	0-100
Extended	0-400	0-100	0-100	-
Frozen	0-1000	0-100	0-100	-

Sensitivity, specificity, precision and correlation to manual method established in the in-house and field clinical trials using equine semen samples.

Clinical claims:

Sensitivity
- Concentration: 90%
- Motility: 90%
- Prog. Motility: 90%

Specificity
- Concentration: 90%
- Motility: 90%
- Prog. Motility: 80%
- Morphology: 85%

Precision (Intra-device CVs)
- Conc.: 3%
- Motility: 3%
- Prog. Motility: 7%
- Morphology: 3%

Precision (Inter-device CVs)
- Conc.: 10%
- Motility: 10%
- Prog. Motility: 10%
- Morphology: 10%

Accuracy (regression coefficients of the dilution trend line)
- Conc.: 0.9
- MSC: 0.9

Table 2. Sensitivity/Specificity

SQA-Ve vs. Microscope	Sensitivity %	Specificity %	% False Positive	% False Negative
Sperm Concentration	96.4	100.0	0	2.2
Motility	95.0	96.3	2.1	2.1
Progressive Motility	100.0	90.0	4.3	0
Morphology	-	93.3	6.3	6.3

Table 3. Precision: SQA-Ve intra- and inter-device variability

Semen Parameters	Intra-device CV, %	Inter-device CV, %
Sperm Concentration	2.0	7.0
Motility	0.3	7.2
Prog. Motility	5.6	8.6
Morphology	0.3	2.6

Correlation to Manual Method

- Concentration: 0.9
- Motility: 0.9
- Prog. Motility: 0.8
- Morphology: 0.7

Notes:

o *Sensitivity and specificity claims are lower than actual values noted (Table 2).*

o *Precision CV claims are higher (lower precision) than actual values noted (Table 3).*

o *Correlation to Manual Method claims are less than actual correlations noted (Table 4).*

Method comparison:
The SQA-Ve was compared to the microscope based on WHO'99 manual guidelines. The SQA-Ve automated readings for sperm concentration, motility, progressive motility and morphology were compared to microscopic results. A Makler chamber was used according to the manufacturer's instructions for manual sperm concentration measurements. A microscope and standard slide were used to manually assess motility. Stained slides were used for the manual morphology examination. The protocols were based on WHO'99 manual and MES guidelines. The clinical trials were conducted at the Medisoos vet clinic. A total of 201 raw, extended, cooled and frozen semen samples were analyzed.

Accuracy: Dilution plots.
The accuracy of the SQA-Ve was assessed by diluting equine semen and analyzing the resulting sperm concentrations. Raw stallion semen was gradually diluted with commercial extender. Dilutions provided varying motile and total sperm concentrations. Semen samples were tested using the SQA-Ve and the results were plotted. Linear trend lines were established for Concentration and MSC vs. expected values.

Analytical Specificity:

- To achieve analytical specificity a specific wave length of light which is maximally absorbed by sperm cells and minimally absorbed by other cells and seminal plasma is used.
- Low noise and high electronic resolution hardware components and compensation circuits ensure analytical specificity optimization.

Limitations of method:
Samples were assessed in duplicate on automated SQA-Ve systems and manually using a microscope. Statistical counting errors and intra-operator variability (subjectivity) may have affected the results of the study.

Table 4: Correlation to Manual Method

Semen Parameters	Correlation coefficients
Sperm Concentration, M/ml	**0.996**
Motility, %	**0.956**
Progressive Motility, %	**0.892**
Morphology, %	**0.744**

Fig. 1. Method comparison: Regression plot of SQA-Ve Sperm Concentration in Raw equine semen vs. manual results

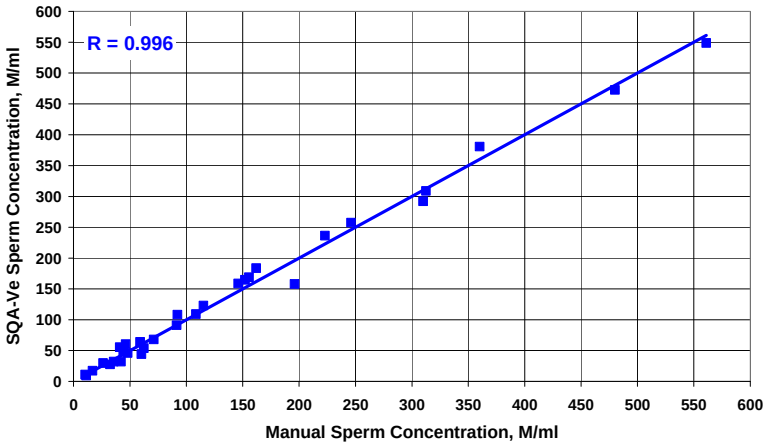
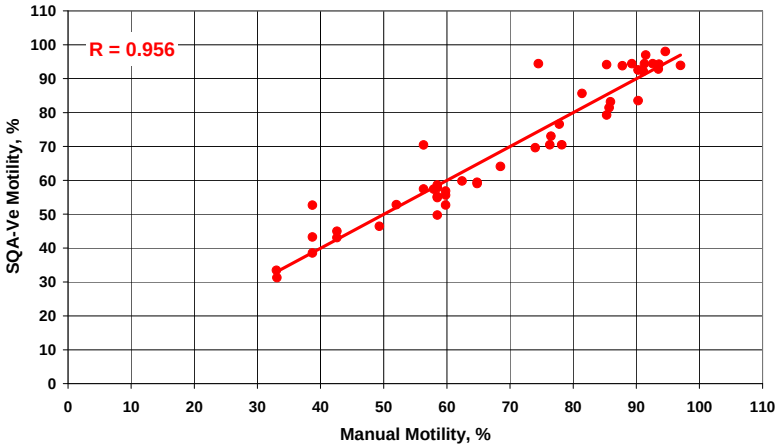


Fig. 2. Method comparison: Regression plot of SQA-Ve Motility in Raw equine semen vs. manual results



Performance parameters:

- Sensitivity and specificity were calculated using ROC analysis. Cutoffs normally used for sperm concentration, motility and morphology were used for the calculation of sensitivity, specificity, false positive and false negative parameters (Table 2).
- Precision of the SQA-Ve was estimated by calculation of the intra-device and inter-device coefficients of variation (CV) of duplicate measurements (Table 3). CV is calculated according to the formula:

CV = SD / MEAN x 100

The lower CV, the higher precision of the method.

- Correlation to manual method was established by calculating correlation coefficients (Table 4, Fig. 1-3).
- The accuracy of the SQA-Ve was determined by the regression coefficients of the dilution trendline (Fig. 4).

Fig. 3. Method comparison: Regression plot of SQA-Ve Morphology in Raw equine semen vs. manual results

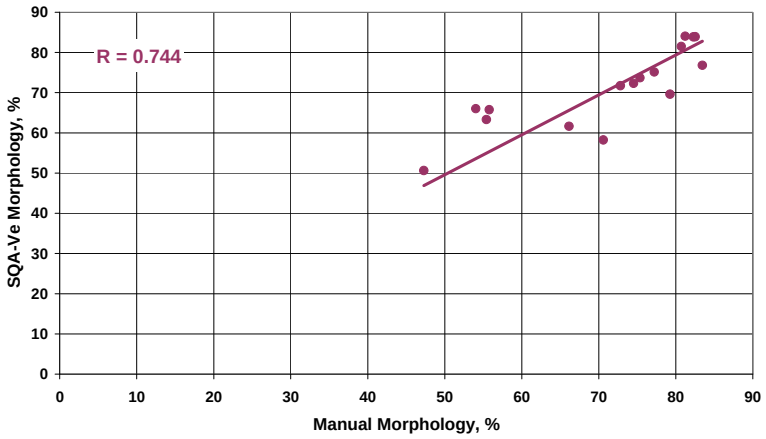
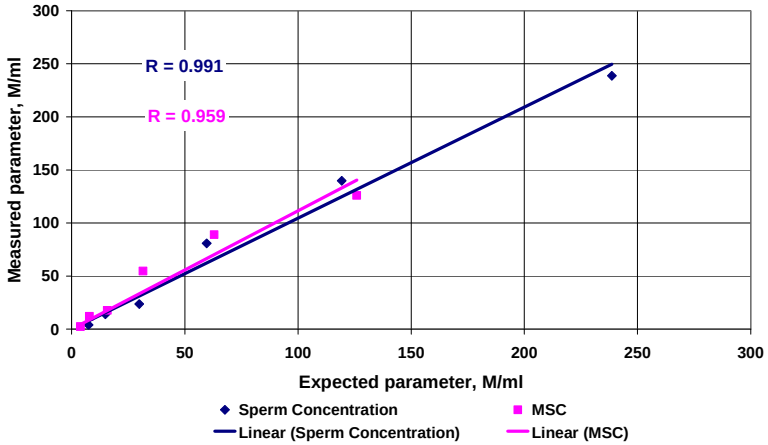


Fig. 4. Regression plot of SQA-Ve Conc. & MSC in Extended equine semen vs. expected values



Conclusions:

- *The SQA-Ve demonstrated high levels of sensitivity, specificity and correlation to the manual method.*
- *The SQA-Ve is precise and accurate with low coefficients of variation for all semen parameters assessed (<10%).*
- *The SQA-Ve can be used for semen quality assessment, dose preparation and to QC frozen equine semen.*