Appendix VIII: Glossary of Terms

	SQA-Vp Terms	Definition
Menu	SN	Serial Number of the SQA-Vp
	DATE/TIME	The date and time the test was performed
	SAMPLE #	The number assigned to the semen sample
	BOAR ID	The identifying number of the boar being tested
Test Results	CONC.	Total sperm concentration expressed in millions/ml
	MSC	Motile sperm concentration expressed in millions/ml
	MOTILITY %	Percentage of motile spermatozoa: Motile Sperm Concentration divided by Total Sperm Concentration expressed as a %.
	GRADING MOTILITY	Motility Grade score (0-5) is related to progressiveness of motile sperm. Score 0 corresponds to absence of progressive motility whereas score 5 corresponds to the maximum progressive motility.
	MORPHOLOGY	Percentage of morphologically normal spermatozoa.
	SPERM #	The total number of sperm cells per ejaculate volume (Fresh samples) or per semen volume (Extended samples).
	MOT. SPERM	The total number of motile sperm cells per ejaculate volume (Fresh samples) or per semen volume (Extended samples).
Dosing Calculations P-Sperm	EXTENDER VOLUME	The amount (ml) of extender to add to the semen in order to produce the desired number of doses.
	TOTAL VOLUME	Semen volume (neat ejaculate + primary extender volume) + extender volume (ml)
	NUMBER OF DOSES	The total number of doses that will be produced based upon the users set-up parameters
Dosing Set-up P-Sperm	DOSING METHOD	Options to dose by: Total Sperm #; Motile Sperm #; or Morphologically Normal Sperm # in an A.I. dose
	DOSE VOLUME	The desired A.I. dose volume (ml): 80/100/other
	TARGET # SPERM	The number of spermatozoa according to the dosing method desired in an A.I. dose (Billion/Dose)
	MOTILITY CUTOFF	The lowest acceptable level of Motility
	MOTILITY GRADE CUTOFF	The lowest acceptable level of Motility Grade

Appendix IX: SQA-Vp 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, DELETE, ENTER, four cursor buttons, ESC, numeric buttons (0-9)
- **Video control keys**: ON/OFF, 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)

Archive Capacity

- 500 test records in each (Test Results and Control) archive

Operating System

- **Control:** Keypad
- **Analysis Time:** 45 seconds for a Normal Test
- **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:** 37^0C (98.6^0F).
- **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.
- **External:** QwikCheck-beads control media.

PC Compatibility

Minimum requirements for P-Sperm™ software

- **PC:** 1 GHz processor, Pentium 3
- **RAM:** 256 MB
- **AGP-video display card** with at least 16 MB of RAM memory
- **Video color**: At least 16 bit (65,535)
- **CD ROM drive**
- **40 GB free hard disk space** for image capturing and storage
- **Video resolution:** Minimum 640 x 480

Operating system compatibility: Windows 2000, Windows XP, DirectX, DivX

- **Ports:** One serial; two USB ports
- **Monitor:** 15" color

Additional Software (supplied with system)

- **P-Sperm software:** Real time visualization interface between PC and SQA-Vp visualization system, data transfer, video/picture capture and archive.

Operational Temperature and Humidity

- System is operational at 15-38°C.
- *NOTE*: SQA-Vp operates in a wide range of ambient temperatures however the system is calibrated to measure semen samples at 37^0C (98.6^0F).
- 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-Vp 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

Sample Type: FRESH

Automatically print: YES

Display test results: YES

Automatically send to PC: YES

Extender transparent: YES

Appendix X: Product Performance Data

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

Performance Data Summary:
The performance of the SQA-Vp system for boar semen analysis is summarized in the text, tables and graphs below. Sperm concentration measurements are expressed as 10^6 sperm cells per milliliter (M/ml). Motility is expressed as a percent (%). Unless otherwise noted all testing was performed using fresh and extended boar semen samples.

Calibration:
Each SQA-Vp is biologically calibrated against two reference systems at Medical Electronic System's laboratory using boar semen.

Dynamic Range:

Sample Type	Conc. M/ml	Motility %	Grading Motility	Morphology %
Fresh	0-1500	0-100	0-5	0-100
Extended	0-500	0-100	0-5	-

Precision and accuracy established against a known target (Latex beads)

Background: The precision and accuracy of the SQA-V were compared to a known target value using commercially available latex beads of two concentrations. Latex beads are used commercially to validate automated sperm counting systems. The beads were run on the SQA-V in the same manner semen samples are run on the system.

Limitations of method:
Latex beads cannot:
- Measure sperm motility or morphology
- Correct for inaccurate chamber depths or technician errors

Methodology:
A total of 320 latex bead samples were tested on ten SQA-V systems. The precision of the SQA-V is demonstrated in Table 1. SQA-V concentration readings were compared to the established target values +/- the acceptable range for the latex beads (Fig. 1 & 2).

Accu-beads® published ranges (*Hemacytometer*):
- Vial #1: 47 +/- 7.0 M/ml
- Vial #2: 24 +/- 3.4 M/ml

Table 1: Precision

SQA-V	Latex-beads	CV %
Intra-device Variability	**High 47± 7.0 M/ml**	**≤ 0.01**
	Low 24 ± 3.4 M/ml	**≤ 0.01**
Inter-device Variability	**High 47± 7.0 M/ml**	**≤ 2.00**
	Low 24 ± 3.4 M/ml	**≤ 2.50**

Fig. 1. Accuracy: High Level Control

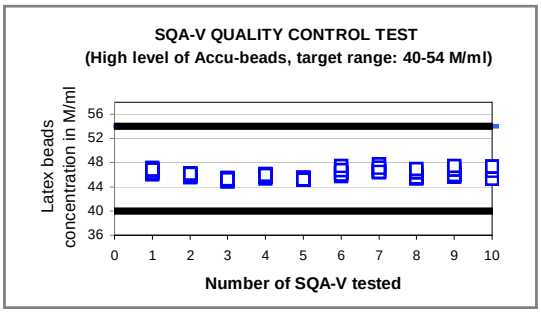
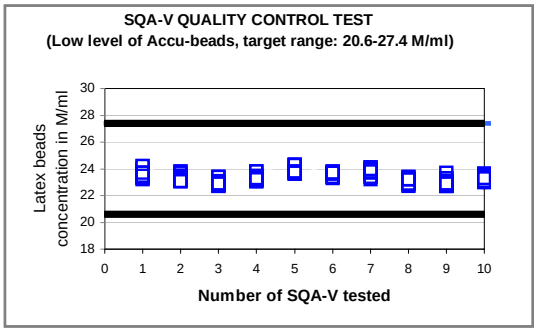


Fig. 2. Accuracy: Low Level Control



Conclusions:

The **CONTROL** mode software of the SQA-Vp (pig) device is exactly the same as the SQA-V (human) system. Both systems also have the same hardware platform. Therefore, the accuracy and precision results obtained on the CONTROL mode of the SQA-V will be the same as that of the CONTROL mode of the SQA-Vp.

Sensitivity, specificity, precision and correlation to manual methods established in MES laboratories and field clinical trials using boar semen samples

Clinical claims:

Sensitivity

- Concentration: 90%
- Motility: 75%
- Grading Motility: 60%
- Morphology: 60%

Specificity

- Concentration: 90%
- Motility: 75%
- Grading Motility: 80%
- Morphology: 80%

Precision (CVs)

- Conc.: 3%
- Motility: 5%
- Morphology: 5%

Correlation to Manual Method:

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

Table 2: Sensitivity/Specificity

SQA-Vp vs. Microscope	Sensitivity, %	Specificity, %
Sperm Concentration	**91.7**	**95.2**
Motility	**80.0**	**79.0**
Grading Motility	**66.7**	**89.1**
Morphology	**66.7**	**84.0**

Table 3: Precision: SQA-Vp intra-device variability (CV, %)

Semen Variable	CV, %
Sperm Concentration M/ml	**2.1**
Motility, %	**4.0**
Morphology, %	**3.6**

Notes:

- Sensitivity and specificity **claims** are lower than actual values noted (Table 2).
- Precision CV **claims** are higher (lower precision) than actual values noted (Table 3).
- Correlation to Manual Method **claims** are less than actual correlations noted (Table 4).

Method comparison:

SQA-Vp was compared to the microscope based on WHO'99 manual guidelines. The SQA-Vp automated readings of the sperm concentration, motility and morphology were compared to microscopic results. A Makler chamber was used according to manufacturer's instructions for manual sperm concentration measurements. A standard slide and P-Sperm software were used to assess manual motility. The stained slides were used for the manual morphology examination. The protocols were based on WHO'99 manual and MES guidelines. The alpha-site clinical trials were conducted at the Lahav farm. A total of 58 fresh and extended semen samples were analyzed.

Table 4: Correlation to manual method

Parameters	Correlation coefficients
Sperm Concentration, M/ml	**0.99**
Motility, %	**0.83**
Morphology, %	**0.71**

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 that analytical specificity is optimized.

Limitations of method:

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

Performance parameters:

- Sensitivity and specificity were calculated using ROC analysis formulas. The cutoffs normally used for the sperm concentration, motility and morphology were used for calculation of sensitivity and specificity (Table 2).
- Precision of the SQA-Vp device was estimated by calculation of the intra-device coefficients of variation (CV) of the 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 calculation of correlation coefficients (Table 4, Fig. 3-5).

Fig. 3: Method comparison: Regression plot of SQA-Vp Sperm Concentration in boar semen vs. manual results

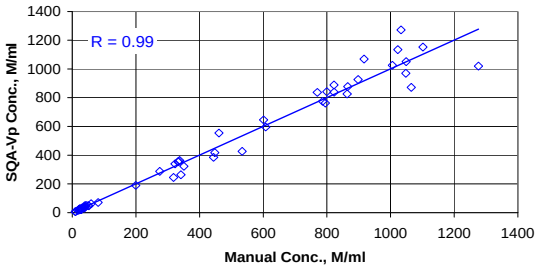


Fig. 4: Method comparison: Regression plot of SQA-Vp Motility in boar semen vs. manual results

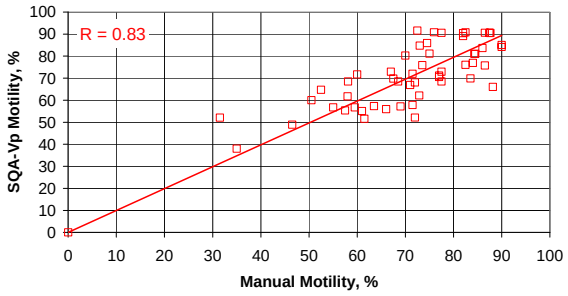
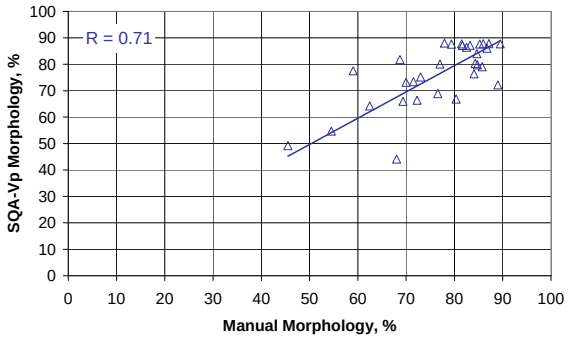


Fig. 5: Method comparison: Regression plot of SQA-Vp Morphology in fresh boar semen vs. manual results



Conclusions:

- The sensitivity, specificity and correlation of the SQA-Vp to the manual method are very high. Therefore the instrument can replace the manual method for assessing, dosing and extending pig semen.
- The SQA-Vp is precise and reports accurate results with low coefficients of variation for the semen variables assessed (<6%).