



Appendix IX: 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 10⁶ 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 (Hemocytometer):

- Vial #1: 47 +/- 7.0 M/ml
- Vial #2: 24 +/- 3.4 M/ml

Table 1: Precision

SQA-V	Accu-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

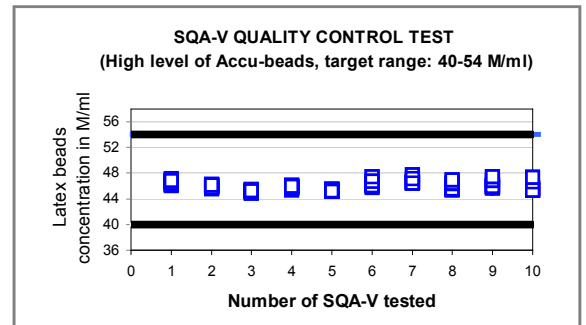
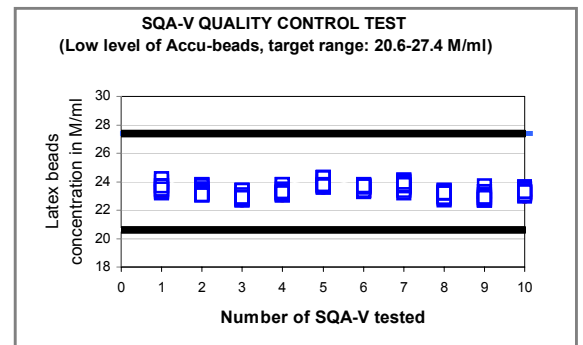


Fig. 2. Accuracy: Low Level Control



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

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 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

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:

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

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).

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%).

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

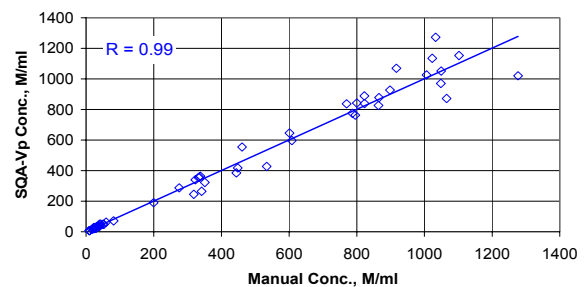


Fig. 4: Method comparison: Regression plot of SQA-Vp Motility in boar semen vs. manual results

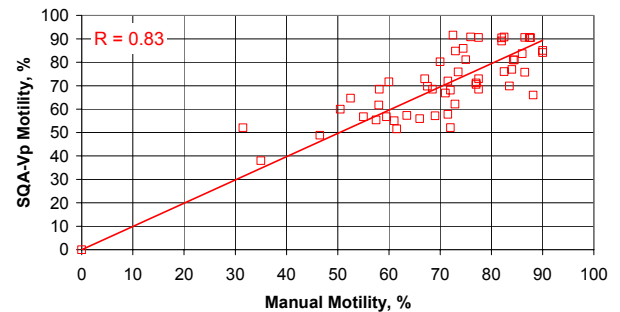


Fig. 5: Method comparison: Regression plot of SQA-Vp Morphology in fresh boar semen vs. manual results

