Appendix X: Product Performance Data

Abbreviations:

CONC:	Sperm Concentration
MSC:	Motile Sperm Concentration
PMSC:	Progressively Motile Sperm Concentration
Mic.	Micron
Sec.	Second
M/ml	Million per milliliter

Performance Data Summary:
The performance of the SQA-Vb system for bull semen analysis is summarized in the text, tables and graphs below. Sperm concentration measurements are expressed as $x10^6$ sperm cells per milliliter (M/ml). Motility and Morphology values are expressed as a percent (%). Unless otherwise noted all testing was performed using fresh and frozen bull semen samples.

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

Dynamic Range:

Sample Type	Test Mode	Conc. M/ml	Motility %	Morphology %	MSC M/ml	PMSC M/ml	Velocity, mic./sec.
Fresh	Fresh	0-2000	0-95	0-100	0-1900	0-1800	0-130
Frozen	Frozen	0-100	0-95	-	0-950	0-900	0-80

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

Background: The precision and accuracy of the SQA-V was compared to a known target value using commercially available latex beads of two concentrations. Latex beads are run on the SQA-V in the same manner as semen samples.

Limitations of method:
Latex beads cannot:

- Measure sperm motility or morphology
- Correct for inaccurate chamber depths or technician errors

Method comparison:
A total of 320 latex bead samples were tested on ten SQA-V systems (32 samples per SQA-V). Precision of the SQA-V was estimated (Table 1). SQA-V concentration readings were compared to the established target values +/- the acceptable range for the latex beads (Fig. 1 & 2).

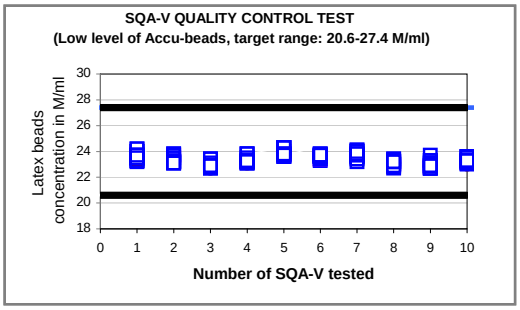
Accu-beads® published acceptable ranges (*Hemacytometer*):

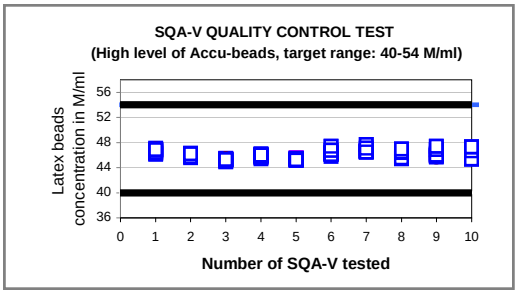
- Vial #1: 47 +/- 7.0 M/ml
- Vial #2: 24 +/- 3.4 M/ml

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- 2 Accuracy
Low/High Level Controls:





Conclusions:

The CONTROL mode software of the SQA-Vb (bull) 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-Vb.

Sensitivity, specificity, precision, accuracy and correlation to manual method established in the MES laboratory and field clinical trials using bull semen samples

FRESH SEMEN Performance Claims (Table 2-5)

Sensitivity

- Concentration: 90%
- Motility: 85%
- Morphology 80%

Specificity

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

Precision (CVs)

- Conc.: 3%
- Motility: 5%
- MSC: 7%
- Velocity: 10%

Accuracy (regression coefficients of "live/dead" trend line) Claims:

- Motility: 0.85
- MSC: 0.9
- PMSC: 0.9

Correlation to Manual Method Claims:

- Concentration: 0.9
- Motility: 0.8
- Morphology 0.7
- MSC: 0.9
- PMSC: 0.8
- Velocity: 0.75

FROZEN SEMEN Performance Claims (Table 3, 5)

Precision (CVs)

- MSC: 7%
- Velocity: 4%

Correlation to Manual Method

- MSC: 0.8
- PMSC: 0.7
- Velocity: 0.85

Table 2: Sensitivity/Specificity

SQA-Vb vs. Microscope	Sensitivity	Specificity
FRESH SEMEN		
Sperm Concentration M/ml	**100.0%**	**98.4%**
Motility, %	**96.3%**	**85.0%**
Morphology	**85.7%**	**91.7%**

Table 3: Precision
SQA-Vb intra-device Variability (CV, %)

Parameter	Sample Type	
	Fresh	Frozen
Sperm Concentration M/ml	**2.4**	**-**
Motility, %	**4.1**	**-**
Morphology %	**5.0**	**-**
MSC, M/ml	**6.1**	**6.4**
Velocity, microns/sec.	**9.9**	**3.4**

Table 4: Accuracy:
Regression coefficients from "live/dead" experiments

Parameter	SQA-Vb	Manual
Motility, %	**0.9135**	**0.8325**
MSC, M/ml	**0.9348**	**0.7547**
PMSC, M/ml	**0.9340**	**-**

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).
- Accuracy regression coefficient claims are less than actual values noted (Table 4).
- Correlation to Manual Method claims are less than actual correlations noted (Table 5).

Method comparison:

SQA-Vb was compared to the microscope based on WHO '99 guidelines. The SQA-Vb automated readings of the sperm concentration, motility, MSC, PMSC and velocity were compared to microscopic results. A Makler chamber was used according to manufacturer's instructions for the manual sperm concentration measurements. A standard slide and B-Sperm software were used to assess manual motility, progressive motility and velocity measurements. Manual MSC and PMSC parameters were calculated from experimental results. The protocols were based on WHO '99 and MES guidelines. The alpha-site clinical trials were conducted at the Sion farm. A total of 104 fresh and 138 frozen semen samples were analyzed.

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-Vb system and manually using a microscope. Statistical counting errors and intra-operator variability (subjectivity) may have affected the results of the study.

Accuracy assessment: "live/dead" sperm plots.

The SQA-Vb accuracy was assessed by "live/dead" bull sperm experiments. Fresh bull semen was distributed into two aliquots. The first aliquot was intact ("live") and the second one was treated with the liquid nitrogen ("dead"). Then different "live-to-dead" proportions were created providing a constant Sperm Concentration but varying MSC, PMSC and Motility. The samples were tested using the SQA-Vb device and under the microscope and the results plotted. The linear trend lines were established for motility, MSC and PMSC variables vs. "Live/Dead" sperm ratio.

Table 5: Correlation to manual method

Parameters	Correlation coefficients	
	Fresh semen	Frozen semen
Sperm Concentration, M/ml	0.93	-
Motility, %	0.81	-
Morphology %	0.71	-
MSC, M/ml	0.94	0.84
PMSC, M/ml	0.86	0.74
Velocity, mic./sec.	0.81	0.91

Fig. 3: Method comparison: Regression plot of SQA-Vb Sperm Concentration in fresh bull semen vs. manual results

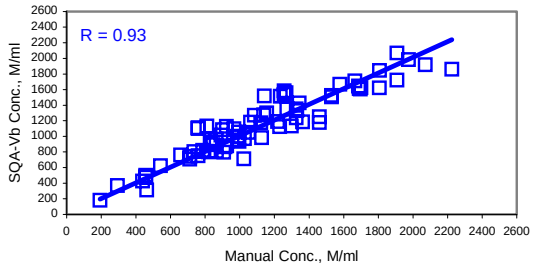


Fig. 4: Method comparison: Regression plot of SQA-Vb Motility in fresh bull semen vs. manual results

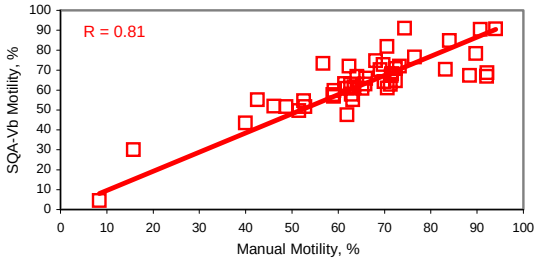
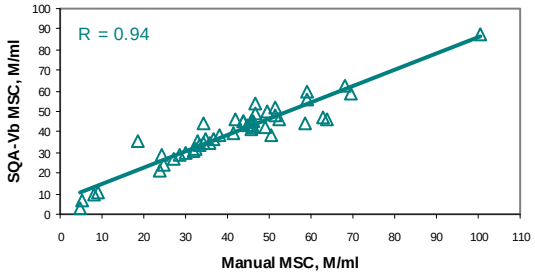


Fig. 5: Method comparison: Regression plot of SQA-Vb MSC in fresh bull semen vs. manual results



Performance parameters:

- Sensitivity and specificity were calculated using ROC analysis formulas. The cutoffs normally used for the sperm concentration and motility of the fresh semen samples were used for calculation of sensitivity and specificity. As for the frozen samples there are no cutoffs, sensitivity and specificity were not calculated for this type of samples (Table 2).
- Precision of the SQA-Vb 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 accuracy of the SQA-Vb device was characterized by regression coefficients of the trendline obtained in the “live/dead” experiment (Table 4).
- Correlation to manual method was established by calculation of correlation coefficients (Table 5, Fig. 3-5).

Conclusions:

- High levels of sensitivity, specificity and correlation of the SQA-Vb device results to the manual method were found. Therefore the instrument can be used in the field for the semen quality assessment, dose preparation and in the frozen semen QC.
- SQA-Vb provides the precise and accurate results with low coefficients of variation (<6%) and high regression coefficients of the “live/dead” trend lines (>0.95).