



CLINICAL REVIEW

SQA-Vp, QwikCheck™GOLD PIG and QwikCheck™QC PORCINE SPERM QUALITY ANALYZERS

Company Overview

Medical Electronic Systems (MES) and Advanced Agricultural Technologies (A-Tech – Veterinary Division) specialize in the development and manufacture of rapid, automated semen analyzers for both the human and the veterinary markets. The Company's comprehensive line of CE and FDA approved Sperm Quality Analyzers includes a specialized line for pigs: SQA™-Vp, QwikCheck™GOLD PIG and QwikCheck™QC Pig – all utilizing the same algorithms. In addition, dosing/data management software, P-Sperm, is included with the top of the line SQA-Vp sperm analyzer. All analyzers and associated testing kits are sold through an extensive network of international distributors.

In 1993, Medical Electronic Systems began manufacturing a unique line of instruments which were able to accurately characterize and rapidly analyze sperm cell concentration, motility and morphology. Continued extensive research in the application of electro-optics and video microscopy resulted in the development of the proprietary algorithms that are the cornerstone of the company's technology. All veterinary sperm analyzers have unique algorithms that were developed for the individual species.

MES is ISO certified as a medical manufacturer and has offices in the U.S., Europe, Hong Kong and Israel. With over 2500 SQA's installed globally, Medical Electronic Systems is committed to providing excellent service and cutting edge technology to our customers, while becoming the industry standard for rapid and reliable automated semen analysis.

Technology

The algorithms in the SQA™-Vp + P-Sperm™, QwikCheck™ GOLD and QwikCheck™ QC Pig Sperm Analyzers were developed to address the specific characteristics of porcine semen (movement, shape and concentration) and the specific needs of the pig reproduction market – including cut-offs, dosing, motility grading, etc. Features such as the QwikClick™ Morphology Counter combine morphology assessment algorithms with image processing to provide fast, precise and accurate morphology results with minimal user interface.

Using a washable (ten use) testing capillary, boar semen samples are analyzed in less than one minute in a temperature (37°C) controlled measurement compartment. The SQA has two independent detection channels: electro-optical and spectrophotometry. These independent channels detect signals from both total and motile spermatozoa. Proprietary algorithms read signals produced by sperm cells and report the following automated test results:

- Sperm Concentration
- Percent Motility
- Motility Grading
- Motile Sperm Concentration
- Normal Morphology
- Number of total sperm per ejaculate
- Number of motile sperm per ejaculate

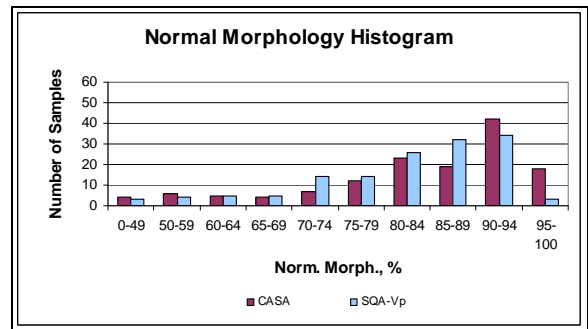
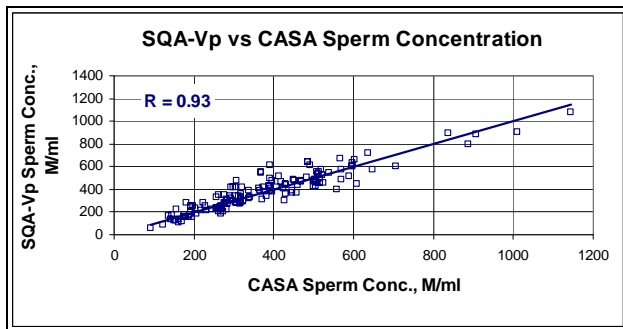
The premiere model of A-Tech analyzers: The SQA-Vp + P-Sperm was developed for high throughput AI centers. This system provides the following features:

- Menu driven operation: Instructional screens lead the operator through the testing/dosing cycle
- In one process, FRESH sperm can be tested and dosing instructions provided for EXTENDING the sample. EXTENDED sperm samples can then be tested for QC.
- Automated dosing can be performed based on: number of total, motile or morphologically normal sperm per dose
- Cost effective multi-use capillaries reduce the cost per test
- QC: Self-testing, Self-calibrating; Runs latex beads QC material
- On-board printer
- PC interface with P-Sperm™ dosing/data management software archives test/dosing results by boar
- Integrated microscope (x300 & x500) with slide adaptor included

Clinical Trials

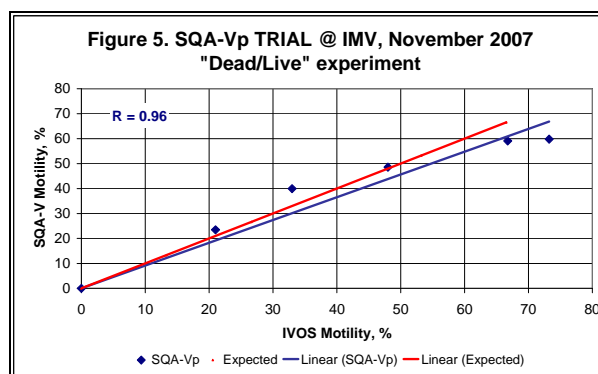
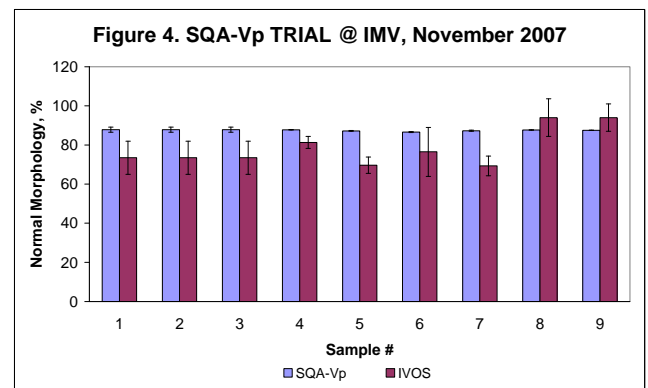
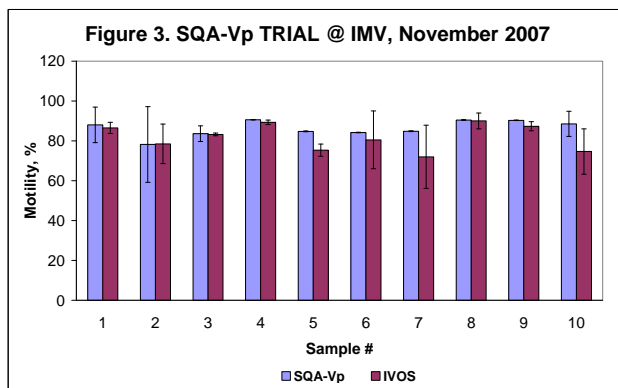
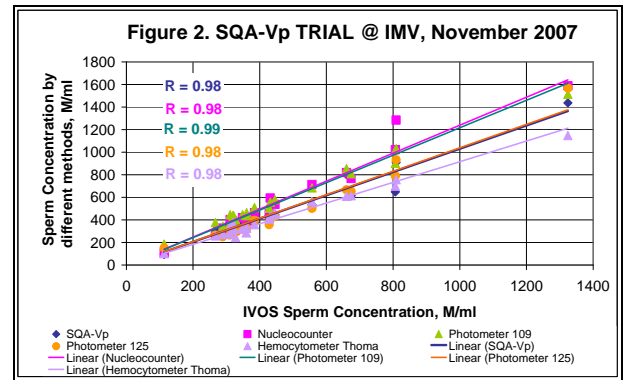
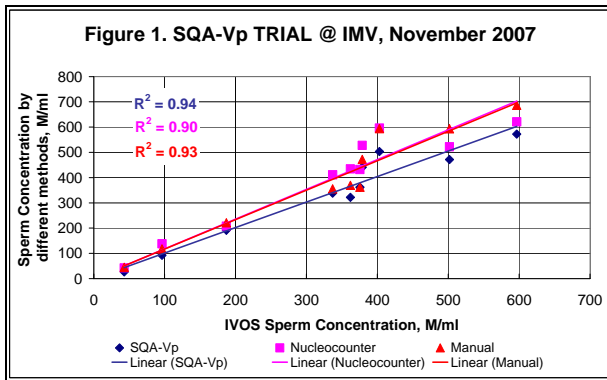
Beginning in March 2007, a number of SQA-Vp clinical trials were conducted at the commercial sites. The unpublished results of these trials are presented in this section.

- **Bear Creek Genetics, LLC, U.S. (December 2008 – February 2009) and (April – May 2009):** A trial was conducted under the supervision of Dr. Chris Kuster comparing the SQA-Vp to CASA at Bear Creek Genetics, LLC, a commercial boar stud, with a subset of samples verified at an independent referral laboratory (KRC). In total, more than 700 semen samples were assessed during three phases of study. Relevant statistical analyses were applied to the data obtained in this trial. The following results obtained using the most updated SQA-Vp software during the third stage of the trial covering 140 fresh boar semen samples are listed below:
 - The SQA-Vp correlation coefficient for Sperm Concentration vs. CASA was 0.93 (manufacturer claim – 0.9).
 - Correlation analysis was not applicable for Motility in this study, as most of the data points were at the upper end of the dynamic range. High Specificity 94.9% (claim – 75%) and Negative Predictive Value 100% were found for Motility.
 - The SQA-Vp Morphology specificity was 80.8% (claim – 80%) and Negative Predictive Value – 88.2% based on the results reported by the SQA-Vp system vs. manual data.
 - High precision of the SQA-Vp was found: Averaged coefficients of variation vs. manufacturer's claims in brackets are: Concentration – 2.1% (3%), Motility – 2.4% (5%) and Morphology – 4.1% (5%).
- **Conclusion:** All SQA-Vp statistical parameters were close or exceeded the manufacturer claims.



The first graph above demonstrates a tight SQA-Vp vs. CASA sperm concentration correlation. The second histogram demonstrates an acceptable Normal Morphology distribution pattern for both the SQA-Vp and CASA systems. The automated SQA-Vp morphology outcome was supplemented with a formula that integrated manual proximal and distal droplets with automated morphology results. This formula is integrated into a feature called QwikClick™ that is now standard in the SQA-Vp software but was not available during the trial. Count and final morphology distribution is presented in the graph. Under the CASA labeling, full manual morphology counting is presented.

- IMV, France (November 2007):** The SQA-Vp (porcine) sperm quality analyzer, nucleocounter (concentration only), two photometers (concentration only) and manual semen analysis were compared to the IVOS (CASA) system. Each device was operated according to the manufacturer instructions. Manual sperm concentration was assessed under the microscope using a Thoma hemacytometer. Fully automated reports including Morphology were generated using the SQA-Vp in less than 1 minute. The IVOS system automatically reported semen parameters except for Morphology and consequently it was found that the test time was increased on the IVOS because of the need to manually assessing this parameter. Sperm concentration, motility, motile sperm concentration and morphology were compared by regression graphs, correlation coefficients and precision (coefficients of variation – CV). A total of 38 fresh and extended semen samples were analyzed. The results of this trial are presented below:

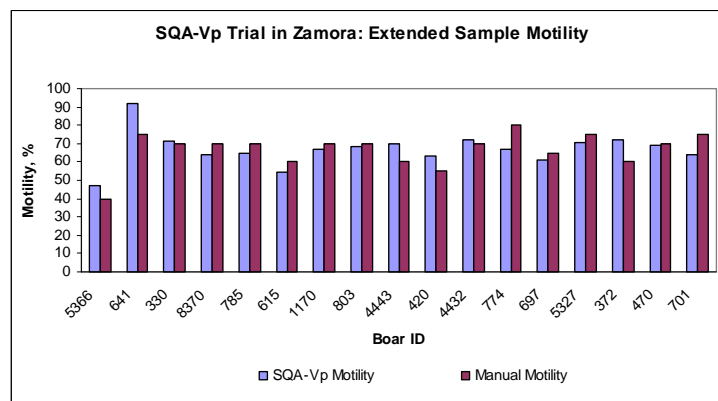


- Precision:** The SQA-Vp demonstrated a CV<3%, better than the other methods. The correlation between the SQA-Vp and IVOS was high (Fig. 1, 2 and 5). Sperm concentration results reported by the nucleocounter, photometers and manual method were highly correlated to the IVOS system, but they were less accurate than SQA-Vp as demonstrated by the fit of the SQA-Vp trendline to the IVOS data points.
- Accuracy:** Typically, boar semen selected for reproduction has optimal Motility and Morphology characteristics (top of the range), therefore, accuracy was assessed using column graphs with 95% Confidence Interval bars (Fig. 3-4). The graphs show quite similar Motility and Morphology results between the SQA-Vp and IVOS, demonstrated by 95% Confidence Interval bars overlapping in most of the samples.

- **Dead/Live:** For this comparison, a wider dynamic range of Motility results was required. Semen was pooled and then divided into two aliquots; one aliquot was intact and the other was treated with liquid nitrogen. As a next step, the semen samples from the different aliquots were mixed in different proportions and analyzed. The results were plotted in a graph (Fig. 5) that demonstrates that the SQA-Vp vs. IVOS trendline is close to the expected – with a regression coefficient of 0.96.
- **Conclusion IMV TRIAL:** The SQA-Vp provides precise results with low coefficients of variation for boar semen (CV=0.3-2.4%) compared to IVOS (CV=4.8-5.8%). A high level of correlation was demonstrated between the SQA-Vp and the IVOS. The SQA-Vp vs. the IVOS demonstrated the best accuracy of any of the methods assessed. The SQA-Vp automated results including Morphology are generated in less than 1 minute – more rapidly than other methods. The SQA-Vp can be used for rapid, precise and accurate boar semen assessment and QC for extended semen.
- **SCHIPPERS, Holland (September-October 2008):** An SQA-Vp vs. UltiMate CASA comparison trial was contracted from an independent lab by Schippers, without training or support from M.E.S./A-Tech. The number of observations **n** is 462 for Concentration and Motility, 400 for Morphology and 140 for Precision. The original data submitted to MES/A-Tech appeared to include some errors (tests with BLANK results were somehow included in the statistical evaluation as were a few samples with obvious sample handling issues). Therefore the raw data was re-calculated by M.E.S. after eliminating the blanks and the sample handling errors. The recalculated Correlation, ROC (Sensitivity & Specificity) and Precision (CV) are presented in the table below:

Semen Parameters	SQA-Vp vs. UltiMate CASA Statistics										
	n	SQA-Vp / UltiMate Mean Diff., %	Correlation		Sensitivity		Specificity		n	Precision	
			r	MES Claim	%	MES Claim	%	MES Claim		CV, %	MES Claim
Sperm Concentration	462	100.2	0.88	0.9	81.9	90	91.9	90	140	4.1	3
Motility	462	95.3	0.80	0.8	81.0	75	83.2	75	140	4.2	5
Morphology	400	93.7			100.0	60	92.8	80	140	4.1	5

- **Mean differences:** SQA-Vp vs. UltiMate mean differences for Concentration (100.2%), Motility (95.3%) and Morphology (93.7%) indicated the absence of the systematic difference between the two systems.
- All SQA-Vp statistical parameters were close or exceeded the manufacturer claims. The SQA-Vp vs. UltiMate Correlation coefficients for Concentration and Motility were high: 0.88 and 0.80 correspondingly. Correlation analysis is not applicable for Morphology in this study, as the dynamic range of this parameter is narrow (only 13 cases of 400 are below 60%). High Correlation and Sensitivity and Specificity of the SQA-Vp vs. UltiMate were demonstrated. The precision of the SQA-Vp was found to be high: Averaged Coefficients of Variation were below 5%. The statistical analysis of this trial demonstrated a high level of agreement between the SQA-Vp and UltiMate.
- **ZAMORA, Spain (March 2007):** FRESH (n=16) and EXTENDED (n=17) boar semen samples were tested using the SQA-Vp, nucleocounter and microscope methods. Correlation of the SQA-Vp sperm concentration vs. nucleocounter was 0.96. The SQA-Vp motile sperm concentration highly correlated with the manual results as well (r=0.92). Dosing results were found to be more consistent using the SQA-Vp + P-Sperm. The SQA-Vp vs. manual Motility in extended samples is presented below:



Conclusions

- In multiple clinical trials conducted worldwide, the SQA-Vp + P-sperm system was compared to a variety of standard methods: CASA, nucleocounter, photometers and microscope.
- In all trials, the SQA-Vp demonstrated high precision and accuracy.
- The coefficients of variation were below 5%.
- The SQA-Vp sperm concentration correlation vs. the other methods was high (>0.9).
- The SQA-Vp live/dead study demonstrated a trendline close to expected.
- The SQA-Vp motility and morphology sensitivity, specificity and negative predictive values were high (varying from 80 to 100%).
- The SQA-Vp Morphology distribution pattern was quite similar to CASA.

References/Articles for both HUMAN and VETERINARY SQA's

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