### Appendix VIII: Glossary of Terms

<table>
<thead>
<tr>
<th>SQA-Vp Terms</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Menu</strong></td>
<td></td>
</tr>
<tr>
<td>SN</td>
<td>Serial Number of the SQA-Vp</td>
</tr>
<tr>
<td>DATE/TIME</td>
<td>The date and time the test was performed</td>
</tr>
<tr>
<td>SAMPLE #</td>
<td>The number assigned to the semen sample</td>
</tr>
<tr>
<td>BOAR ID</td>
<td>The identifying number of the boar being tested</td>
</tr>
<tr>
<td><strong>Test Results</strong></td>
<td></td>
</tr>
<tr>
<td>CONC.</td>
<td>Total sperm concentration expressed in millions/ml</td>
</tr>
<tr>
<td>MSC</td>
<td>Motile sperm concentration expressed in millions/ml</td>
</tr>
<tr>
<td>MOTILITY %</td>
<td>Percentage of motile spermatozoa: Motile Sperm Concentration divided by Total Sperm Concentration expressed as a %.</td>
</tr>
<tr>
<td>GRADING MOTILITY</td>
<td>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.</td>
</tr>
<tr>
<td>MORPHOLOGY</td>
<td>Percentage of morphologically normal spermatozoa.</td>
</tr>
<tr>
<td>SPERM #</td>
<td>The total number of sperm cells per ejaculate volume (Fresh samples) or per semen volume (Extended samples).</td>
</tr>
<tr>
<td>MOT. SPERM</td>
<td>The total number of motile sperm cells per ejaculate volume (Fresh samples) or per semen volume (Extended samples).</td>
</tr>
<tr>
<td><strong>Dosing Calculations</strong></td>
<td></td>
</tr>
<tr>
<td>EXTENDER VOLUME</td>
<td>The amount (ml) of extender to add to the semen in order to produce the desired number of doses.</td>
</tr>
<tr>
<td>TOTAL VOLUME</td>
<td>Semen volume (neat ejaculate + primary extender volume) + extender volume (ml)</td>
</tr>
<tr>
<td>NUMBER OF DOSES</td>
<td>The total number of doses that will be produced based upon the users set-up parameters</td>
</tr>
<tr>
<td>DOSING METHOD</td>
<td>Options to dose by: Total Sperm #; Motile Sperm #; or Morphologically Normal Sperm # in an A.I. dose</td>
</tr>
<tr>
<td>DOSE VOLUME</td>
<td>The desired A.I. dose volume (ml): 80/100/other</td>
</tr>
<tr>
<td><strong>Dosing Set-up</strong></td>
<td></td>
</tr>
<tr>
<td>TARGET # SPERM</td>
<td>The number of spermatozoa according to the dosing method desired in an A.I. dose (Billion/Dose)</td>
</tr>
<tr>
<td>MOTILITY CUTOFF</td>
<td>The lowest acceptable level of Motility</td>
</tr>
<tr>
<td>MOTILITY GRADE CUTOFF</td>
<td>The lowest acceptable level of Motility Grade</td>
</tr>
</tbody>
</table>
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°C (98.6°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.
- **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

- **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°C (98.6°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-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:

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Conc. M/ml</th>
<th>Motility %</th>
<th>Grading Motility</th>
<th>Morphology %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>0-1500</td>
<td>0-100</td>
<td>0-5</td>
<td>0-100</td>
</tr>
<tr>
<td>Extended</td>
<td>0-500</td>
<td>0-100</td>
<td>0-5</td>
<td>-</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Latex-beads</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-device Variability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>47 ± 7.0 M/ml</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>Low</td>
<td>24 ± 3.4 M/ml</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>Inter-device Variability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>47 ± 7.0 M/ml</td>
<td>≤ 2.00</td>
</tr>
<tr>
<td>Low</td>
<td>24 ± 3.4 M/ml</td>
<td>≤ 2.50</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>SQA-Vp vs. Microscope</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Concentration</td>
<td>91.7</td>
<td>95.2</td>
</tr>
<tr>
<td>Motility</td>
<td>80.0</td>
<td>79.0</td>
</tr>
<tr>
<td>Grading Motility</td>
<td>66.7</td>
<td>89.1</td>
</tr>
<tr>
<td>Morphology</td>
<td>66.7</td>
<td>84.0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Semen Variable</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Concentration M/ml</td>
<td>2.1</td>
</tr>
<tr>
<td>Motility, %</td>
<td>4.0</td>
</tr>
<tr>
<td>Morphology, %</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Table 4: Correlation to manual method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Concentration, M/ml</td>
<td>0.99</td>
</tr>
<tr>
<td>Motility, %</td>
<td>0.83</td>
</tr>
<tr>
<td>Morphology, %</td>
<td>0.71</td>
</tr>
</tbody>
</table>
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:
  \[ CV = \frac{SD}{MEAN} \times 100 \]
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%).