Appendix VIII: QwikCheck GOLD Equine Product Performance Data

Performance Data Summary:

The performance of the QwikCheck GOLD Equine Sperm Analyzer is summarized in the text, tables and graphs below. All values concerning sperm concentration measurements are expressed as 10^6 sperm cells per milliliter (M/ml). Motility values are expressed as a percent (%). Unless otherwise noted, all testing was performed using raw, extended and cooled equine semen samples. Manufacturers claims are generally lower than actual performance data. Please also note that Sensitivity & Specificity are clinical screening parameters that demonstrate the accuracy of device. Sensitivity & Specificity results are based on the cutoffs established by Society of Theriogenology.
Each QwikCheck GOLD Equine device is biologically calibrated against two reference systems at MES A-Tech laboratory.

Abbreviations:

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

Table 1. Dynamic Range

Sample Type	Concentration M/ml	% Motility	% Progressive Motility
Raw	0-500	0-100	0-100
Extended	0-50, >50	0-50, >50	0-40, >40
Cooled	0-50, >50	0-50, >50	0-40, >40

Sensitivity, specificity, precision and correlation to manual method established in the in-house and field clinical trials using equine semen samples

Clinical claims:

Sensitivity
- Concentration: 90%
- Motility: 90%
- Prog. Motility: 90%

Specificity
- Concentration: 90%
- Motility: 90%
- Prog. Motility: 80%

Precision (Intra-device CVs)
- Conc.: 3%
- Motility: 3%
- Prog. Motility: 7%

Precision (Inter-device CVs)
- Conc.: 10%
- Motility: 10%
- Prog. Motility: 10%

Accuracy (regression coefficients of the dilution trend line)
- Conc.: 0.9
- MSC: 0.9

Table 2. Sensitivity/Specificity

QwikCheck GOLD Equine vs. Microscope	Sensitivity %	Specificity %	% False Positive	% False Negative
Sperm Concentration	**96.4**	**100.0**	**0**	**2.2**
Motility	**95.0**	**96.3**	**2.1**	**2.1**
Progressive Motility	**100.0**	**90.0**	**4.3**	**0**

Table 3. Precision: QwikCheck GOLD Equine intra- and inter-device variability

Semen Parameters	Intra-device CV, %	Inter-device CV, %
Sperm Concentration	**2.0**	**7.0**
Motility	**0.3**	**7.2**
Prog. Motility	**5.6**	**8.6**

Correlation to Manual Method

- Concentration: 0.9
- Motility: 0.9
- Prog. Motility: 0.8

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:
The QwikCheck GOLD Equine system was compared to the microscope based on WHO'99 manual guidelines. The QwikCheck GOLD Equine automated readings for sperm concentration, motility and progressive motility were compared to microscopic results. The manual sperm concentration measurements were performed using a cell counting chamber according to the manufacturer's instructions. A microscope and standard slide were used to manually assess motility. The protocols were based on WHO'99 manual and MES guidelines. The clinical trials were conducted at the Medisoos vet clinic. A total of 201 raw, extended and cooled semen samples were analyzed.

Accuracy: Dilution plots.
The accuracy of the QwikCheck GOLD Equine system was assessed by diluting equine semen and analyzing the resulting sperm concentrations. Raw stallion semen was gradually diluted with commercial transparent extender. Dilutions provided varying motile and total sperm concentrations. Semen samples were tested using the QwikCheck GOLD Equine device and the results were plotted. Linear trend lines were established for Concentration and MSC (calculated by CONC. x Motility / 100) vs. expected values.

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, debris and seminal plasma is used.
- Low noise and high electronic resolution hardware components and compensation circuits ensure analytical specificity optimization.

Limitations of method:
Samples were assessed in duplicate on automated QwikCheck GOLD Equine systems and manually using a microscope. Statistical counting errors and intra-operator variability (subjectivity) may have affected the results of the study.

Table 4: Correlation to Manual Method

Semen Parameters	Correlation coefficients
Sperm Concentration, M/ml	**0.996**
Motility, %	**0.956**
Progressive Motility, %	**0.892**

Fig. 1. Method comparison: Regression plot of QwikCheck GOLD Equine Sperm Concentration in Raw equine semen vs. manual results

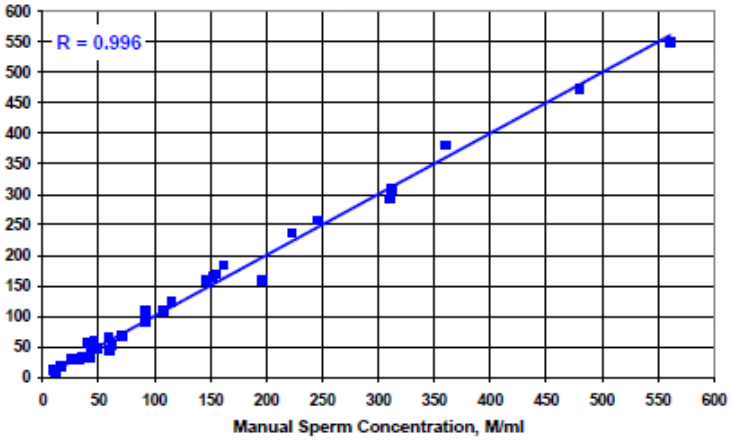
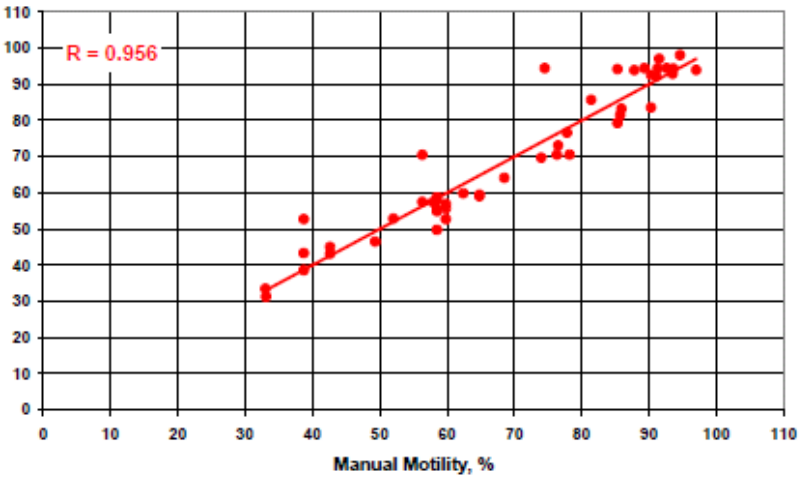


Fig. 2. Method comparison: Regression plot of QwikCheck GOLD Equine Motility in Raw equine semen vs. manual results



Performance parameters:

- Sensitivity and specificity were calculated using ROC analysis. Cutoffs normally used for sperm concentration and motility were used for the calculation of sensitivity, specificity, false positive and false negative parameters (Table 2).
- Precision of the QwikCheck GOLD Equine device was estimated by calculation of the intra-device and inter-device coefficients of variation (CV) of 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 calculating correlation coefficients (Table 4, Fig. 1-2).
- The accuracy of the QwikCheck GOLD Equine system was determined by the regression coefficients of the dilution trendlines (Fig. 3).

Conclusions:

- *The QwikCheck GOLD Equine Sperm Analyzer demonstrated high levels of sensitivity, specificity and correlation to the manual method.*
- *The QwikCheck GOLD Equine system is precise and accurate with low coefficients of variation for all semen parameters assessed (<10%).*
- *The QwikCheck GOLD Equine device can be used for semen quality assessment and QC of equine semen samples.*

Fig. 3. Regression plot of QwikCheck GOLD Equine Conc. & MSC in equine semen vs. expected values