SQA-iO ANALYTICAL STUDIES

Device description

The SQA-iO is a point-of care medical diagnostic device (MDD) designed to perform semen analysis. The device is powered by a computer and accesses cloud based operating software.

1. Accuracy of measurement (trueness, repeatability and reproducibility)

Trial objective

The objective of the study was to establish the trueness, repeatability and reproducibility of the SQA-iO device. Trueness was evaluated based on Correlation, and Positive and Negative Percent Agreement (PPA & NPA) vs. the SQA-V predicate. Precision (repeatability and reproducibility) was assessed based on the WHO 5th edition manual guidelines that define precision as the degree of agreement between duplicate measurements of semen samples. The within-device (repeatability) and between-device (reproducibility) coefficients of variation (%CVs) were established by evaluating duplicate measurements of multiple semen samples.

Reference device used

Sperm Quality Analyzer SQA-V (PREDICATE or SQA)

Trial overview and product claims (acceptance criteria)

"Trueness" is related to the true value and correlation obtained vs. a type of standard. "Precision" of a given measurement procedure is subdivided according to the specified precision conditions. "Repeatability" relates to essentially unchanged conditions and is often termed "within-series" or "within-run precision." "Reproducibility" relates to changes in conditions, eg, time, different laboratories, operators, and measuring systems (including different calibrations and reagent batches) (ISO 17511).

A study was conducted at MEDICAL ELECTRONIC SYSTEMS, ISRAEL (MES) that encompassed each of these methods of evaluation. Four SQA-iO vs. two SQA (PREDICATE) devices were compared in both normal and low volume modes for trueness, repeatability and reproducibility. Accuracy was analyzed using 24 donor semen samples tested in duplicate using normal mode and 23 semen samples tested on the low volume mode. A total of 564 tests were run in this study.

Acceptance criteria per manufacturer product claims:

1. Trueness:
   - PPA & NPA >= 90%
   - Correlation (at least):
     - ✓ Concentration: 0.90
     - ✓ Motility: 0.80
     - ✓ Progressive Motility: 0.80
     - ✓ Morph. Norm Forms (WHO 5th): 0.45

2. Repeatability & Reproducibility:
    - Full volume mode repeatability: CV <= 15%
    - Full volume mode reproducibility: CV <= 15%
    - Low volume mode repeatability: CV <= 20%
    - Low volume mode reproducibility: CV <= 20%

Study protocol

Materials and equipment:

- SQA predicate devices
- SQA-iO devices – 4 units
- Human semen samples
- Test capillaries
- QwikCheck Liquefaction, WBC/pH Test Strips and Dilution Kits

SQA and SQA-Io Settings:

- Conc. standard: 2
- LES: 2
- SW ver: WHO 5th

Semen samples were tested according to the WHO 5th edition manual guidelines by establishing trueness, repeatability and reproducibility parameters based on testing multiple duplicates. To assess the dynamic range of the systems and to ensure a sufficient amount of data above and below the reference cutoffs, approximately 10% of the semen samples were manipulated by centrifuging and diluting part of the cell pellet in seminal plasma. The following WHO 5th reference cutoffs were used:

- Concentration: 15 M/ml
- Motility: 40%
- Progressive Motility: 32%
- Morphology: 4%

Procedure:

1. Collect the semen samples per WHO 5th manual laboratory procedure.
2. Mark each sample with the original sample #.
3. Test sample for WBC and pH using the QwikCheck Test Strips (before step #4).
4. Test only completely liquefied semen samples using QwikCheck Liquefaction kit if needed.
5. Mix each sample thoroughly before proceeding.
6. Test samples in duplicate using four SQA-iO device and two PREDICAT devices.
7. Fill two capillaries with the same semen sample and run in all devices in two opposite directions to get duplicate tests on each device and to compensate for the impact of testing time on motility.
8. Run semen samples in the Fresh full and low volume (10-microliter) mode in duplicate.
9. Save results in the SQA and SQA-iO archives.

Note: Sample #13 and the Progressive Motility replicate from sample #27 were excluded as outliers deviating substantially from the general data trend.

Statistical methods

The following statistical methods were used:

- PPA and NPA – Excel.
- Correlation – MedCalc stats software (Belgium).
- Repeatability & Reproducibility CV – Excel.

Study results

Trueness: The SQA-iO full volume mode trueness was established based on correlation with the SQA PREDICATE results and positive & negative percent agreement (PPA & NPA correspondingly), calculated as follows:

- PPA = P x (P + N) for positive cases by PREDICATE and NPA = N x (N + P) for negative cases by PREDICATE
- Where: P – positive result N – negative result

WHO 5th reference values were used to distinguish between positive and negative results. Accuracy results are shown in Table 1 below.

Table 1. SQA-iO vs. SQA PREDICATE correlation, PPA & NPA results (Full volume mode).

SQA-iO vs. SQA PREDICATE.	Correlation, r	MES claim	PPA	NPA	MES claim
Concentration, M/ml	1.00	0.90	100.0%	100.0%	90.0%
Total Motile PR + NP, %	0.94	0.80	95.8%	100.0%	90.0%
Progressive PR, %	0.98	0.80	100.0%	100.0%	90.0%
Normal Morphology, %	0.99	0.45	90.8%	100.0%	90.0%

The data presented in the Table 1 indicate that the SQA-iO correlation with SQA PREDICATE results, PPA & NPA exceed the MES claims.

Repeatability and Reproducibility: The SQA-iO full volume mode repeatability and reproducibility were evaluated following WHO 5th ed. manual guidelines which recommend testing duplicate samples. The repeatability and reproducibility results assessed via coefficients of variation (CV) calculated between the duplicates of the same device (repeatability) and between results of the different devices (reproducibility) are shown in the Table 2.

Table 2. SQA-iO & SQA PREDICATE Repeatability & Reproducibility (Full volume mode).

Parameters	SQA PREDICATE			SQA-iO repeatability					SQA-iO reproducibility
	Mean	Repeatability CV, %	Reproducibility CV, %	Mean	Lab1 CV, %	Lab2 CV, %	Lab3 CV, %	Lab4 CV, %	CV, %
Concentration, M/ml	55.6	5.3	5.3	54.7	6.1	5.9	6.4	5.8	9.9
Total Motile PR + NP, %	48.5	7.2	4.7	50.5	4.6	7.1	4.3	9.3	12.7
Progressive PR, %	35.6	11.4	13.3	37.4	7.0	6.0	6.8	12.4	14.6
Normal Morphology, %	9.4	6.0	4.9	9.5	5.1	5.2	6.8	11.9	8.6

The mean values of the SQA-iO and SQA PREDICATE systems are quite close demonstrating an absence of systematic discrepancies between devices. The repeatability and reproducibility CV results of all semen parameters are <15%.

The SQA-iO low volume mode accuracy was assessed the same way as the full volume mode. The SQA-iO low volume mode accuracy results are shown in the Table 3.

Table 3. SQA-iO vs. SQA PREDICATE correlation, PPA & NPA results (Low volume mode).

SQA-iO vs. SQA PREDICATE	Correlation, r	PPA	NPA
MSC, M/ml	1.00	100.0%	100.0%
PMSC, M/ml	1.00	100.0%	100.0%

There are no MES claims established for MSC and PMSC. However, the results presented in Table 3 show that the SQA-iO low volume mode PPA & NPA are all equal to 100%. Correlation with SQA PREDICATE results is also optimal (r = 1.0 for both MSC and PMSC).

The most clinically important semen parameters reported by the SQA-iO low volume mode are motile sperm concentration (MSC) and progressively motile sperm concentration (PMSC). The repeatability and reproducibility of these semen parameters were assessed via coefficients of variation (CV) calculated between semen sample duplicates of the same SQA-iO device (repeatability CV) and between the results of different devices (reproducibility CV). Repeatability and reproducibility results of the SQA-iO low volume mode are shown in the Table 4.

Table 4. SQA-iO & SQA PREDICATE Repeatability & Reproducibility (Low volume mode).

Parameters	SQA PREDICATE			SQA-iO repeatability					SQA-iO reproducibility
	Mean	Repeatability CV, %	Reproducibility CV, %	Mean	Lab1 CV, %	Lab2 CV, %	Lab3 CV, %	Lab4 CV, %	CV, %
MSC, M/ml	35.5	9.7	5.6	35.2	7.9	8.2	13.2	12.1	17.0
PMSC, M/ml	24.0	13.2	9.8	29.5	10.3	11.1	16.2	19.4	19.2

The mean values of the SQA-iO and SQA PREDICATE systems are quite close, indicating an absence of systematic discrepancies between these devices in the low volume mode. The repeatability and reproducibility CVs are < 20%.

Study conclusions

- Correlation coefficients between the SQA-iO and the SQA PREDICATE devices are all >= 0.9 exceeding the MES claims for all semen variables.
- Agreement is demonstrated by PPA and NPA of SQA-iO vs. PREDICATE devices exceeding the MES claims of 90%.
- SQA-iO full volume mode repeatability and reproducibility CVs are < 15% (in line with the MES proposed claims).
- SQA-iO lov volume mode repeatability and reproducibility CVs are < 20% (in line with the MES proposed claims).

References

- WHO laboratory manual for the examination and processing of human semen - 5th ed., World Health Organization 2010.
- CLSI EP12-A2, Vol. 28, No. 3, 2008.

2. Analytical sensitivity & specificity

Trial objective

The objective of the study was to establish the analytical Sensitivity and Specificity of the SQA-iO device. The SQA-iO is a point-of care medical diagnostic device (MDD) designed to perform semen analysis. Because it is not an assay, analytical Sensitivity and Specificity were evaluated vs. SQA-V predicate using the reference cutoffs provided by the WHO 5th edition manual.

Reference device used

Sperm Quality Analyzer SQA-V (PREDICATE).

Trial overview and product claims (acceptance criteria).

Analytical Sensitivity is determined by the ability of the tested device to correctly detect abnormal cases vs. the standard method (PREDICATE). Analytical Specificity is determined by the ability of the tested device to correctly detect normal cases vs. the standard method (PREDICATE). The study was conducted at MEDICAL ELECTRONIC SYSTEMS (MES, Israel). Four SQA-iO vs. two SQA (PREDICATE) devices were compared in both normal and low volume modes for Analytical Sensitivity and Specificity.

Acceptance criteria per manufacturer product claims:

Analytical Specificity (at least):

- Concentration: 85%
- Motility: 80%
- Progressive Motility: 80%
- Morph. Norm Forms (WHO 5th): 90%

Analytical Sensitivity (at least):

- Concentration: 90%
- Motility: 85%
- Progressive Motility: 85%
- Morph. Norm Forms (WHO 5th): 80%

Study protocol

Semen samples were tested according to the WHO 5th edition manual guidelines by establishing analytical Sensitivity and Specificity based on testing multiple semen samples. In order to cover the dynamic range of the devices and to ensure a sufficient amount of data above and below the reference value cutoff, approximately 10% of the semen samples were manipulated by centrifuging and diluting part of the cell pellet in seminal plasma. The following WHO 5th reference cutoffs were used to establish Sensitivity and Specificity:

- Concentration: 15 M/ml
- Motility: 40%
- Progressive Motility: 32%
- Morphology: 4%

Procedure:

1. Collect the semen samples per WHO 5th manual laboratory procedure.
2. Mark each sample with the original sample #.

3. Test sample for WBC and pH using the QwikCheck Test Strips (before step #4).
4. Test only completely liquefied semen samples using QwikCheck Liquefaction kit if needed.
5. Mix each sample thoroughly before proceeding.
6. Test samples in duplicate using four SQA-iO device and two PREDICATE devices.
7. Fill two capillaries with the same semen sample and run in all devices in two opposite directions to get duplicate tests on each device and to compensate for the impact of testing time on motility.
8. Run semen samples in the Fresh full and low volume (10-microliter) mode.
9. Save results in the SQA and SQA-iO archives.

Note:

- Sample #13 and the Progressive Motility replicate from sample #27 were excluded as outliers deviating substantially from the general data trend.

Statistical method

- Sensitivity (Excel)
- Specificity (Excel)

Study results

The SQA-iO full volume mode analytical Specificity and Sensitivity indicating an ability of the system to detect normal and abnormal cases respectively vs. SQA PREDICATE were established using the following formulas:

- Specificity = TN x (TN + FP) x 100%
- Sensitivity = TP x (TP + FN) x 100%

Where:

- TN – true negative (negative result when PREDICATE is negative)
- TP – true positive (positive result when PREDICATE is positive)
- FN – false negative (negative result when PREDICATE is positive)
- FP – false positive (positive result when PREDICATE is negative)

The WHO 5th reference values were used to distinguish between positive and negative results. The SQA-iO full volume mode analytical Sensitivity and Specificity results are shown in the Table 5 below.

Table 5. SQA-iO vs. SQA PREDICATE analytical Sensitivity & Specificity results (Full volume mode).

SQA-iO vs. SQA PREDICATE	Sensitivity, %	MES claim	Specificity, %	MES claim
Concentration, M/ml	100.0%	90.0%	100.0%	85.0%
Total Motile PR + NP, %	95.8%	85.0%	100.0%	80.0%
Progressive PR, %	100.0%	85.0%	100.0%	80.0%
Normal Morphology, %	90.8%	80.0%	100.0%	90.0%

The data presented in Table 5 demonstrates that the SQA-iO analytical Sensitivity and Specificity vs. the SQA PREDICATE exceeds the MES claims.

The SQA-iO low volume mode Sensitivity and Specificity, shown in Table 6, were assessed the same way as the full volume mode.

Table 6. SQA-iO vs. SQA PREDICATE analytical Sensitivity and Specificity results (Low volume mode).

SQA-iO vs. SQA PREDICATE	Sensitivity, %	Specificity, %
MSC, M/ml	100.0%	100.0%
PMSC, M/ml	100.0%	100.0%

There are no claims established for MSC and PMSC semen parameters. However, the results presented in Table 6 show that the SQA-iO low volume mode analytical Sensitivity and Specificity are 100% respectively.

Conclusions

The Analytical Sensitivity and Specificity of the SQA-iO vs. the SQA PREDICATE exceed MES claims or are 100%, respectively demonstrating highest level of Sensitivity and Specificity.

References

- WHO laboratory manual for the examination and processing of human semen - 5th ed., World Health Organization 2010.

3. Measuring range of the assay

Trial objective

The objective of the study was to establish the measuring range of the SQA-iO device. As the SQA-iO is a point-of care medical diagnostic device (MDD) designed to perform semen analysis but not an assay, the measuring range was evaluated by testing the Linearity throughout the dynamic range vs. the SQA PREDICATE.

Reference device used

Sperm Quality Analyzer SQA-V (PREDICATE).

Trial overview and product claims (acceptance criteria). .

The study was conducted at MEDICAL ELECTRONIC SYSTEMS (MES, Israel). Three SQA-iO devices vs. two SQA PREDICATES were used to establish and compare the dynamic range of the device. To accomplish this, eight semen samples representing the dynamic range of the SQA PREDICATE were tested in duplicate on three SQA-iO devices and in parallel on the SQA PREDICATES. This resulted in 80 tests in total.

SQA-iO linearity throughout the entire dynamic range was evaluated utilizing the regression coefficients of the linear trend lines and percent recovery computation vs. expected values established by dilution ratios.

Acceptance criteria per manufacturer product claims:
Average recovery 100% +/- 15%. Linear regression coefficient R >= 0.9.

Study protocol

Materials and equipment:

- Two SQA predicate devices
- SQA-iO devices – 3 units
- Human semen samples
- Testing capillaries
- QwikCheck Liquefaction, Test Strips and Dilution Kits

Settings:

SQA and SQA-iO

- Conc. standard: 2
- LES: 2
- SW ver: WHO 5th

To test the dynamic range of the SQA-iO, pooled donor semen samples were centrifuged and the supernatant (seminal plasma) was decanted to establish a blank sample (sperm concentration = 0 M/ml). The resulting sperm pellet and blank seminal plasma were then used to provide a high-level concentration sample of ~200 M/ml. Sequential dilutions of the high-level sample were performed using seminal plasma and all concentration levels were tested on the three SQA-iO devices and in parallel on two SQA PREDICATE systems.

The results were compared to the expected values, based on the dilution rates. The mathematical recoveries were calculated by the equation of observed results divided by expected, times 100. If the outcome of 0 sample is 0, recovery = 100%, otherwise 0%. The final recovery is an average of all the data points. Linear regression coefficients were established from the best fit trend lines of the plots displaying the actual sperm concentration results vs. expected values.

Procedure:

1. Collect and pool 10 liquefied human donor semen samples according to the WHO 5th manual laboratory procedure. Maintain samples at room temperature.
2. Liquefy samples that remain viscous with QwikCheck Liquefaction kit if needed.
3. Centrifuge pooled samples @3000 rpm X 15 minutes; then separate the cell pellet from seminal plasma.
4. Prepare a high-level sample (Conc. ~200 million/ml) using part of the pellet reconstituted in 1 ml of the seminal plasma (verify the concentration of the high-level sample by testing on the PREDICATE).
5. Use the seminal plasma as the ZERO sample and to sequentially dilute the cell pellet as follows:

Sample #	Original Sample %	% Plasma	Concentration M/ml
1	100	0	200
2	80	20	160
3	60	40	120
4	40	60	80
5	20	80	40
6	10	90	20
7	5	95	10
8	0	-	0

6. Mark each semen sample with the **Sample #** that corresponds with the dilution as noted in the table.
7. Thoroughly mix each aliquot before testing and run in duplicate on the three SQA-iO devices and two SQA PREDICATES.

Testing procedure and analysis:

1. **SQA-iO device**: Load the test capillary with the semen sample and follow the SQA-iO instructions.
2. **SQA-V reference PREDICATES**: Load the test capillary with the semen sample and follow the SQA on-screen instructions.

3. Test each sample in duplicate in each SQA-iO device and SQA PREDICATES. Results will be automatically archived.
4. Plot the results vs. expected values calculated based on dilution rates and compute the overall percent recovery and regression coefficient.

Statistical method (method of data analysis)

Regression analysis for establishing the average Recovery throughout the device measuring range (measuring result / expected value x 100%) and Linear Regression Coefficient generated from the trend line were analyzed by Excel.

Study results

The SQA-iO linearity across the device measuring range was established by testing sequential dilutions on 3 SQA-iO and two PREDICATE devices based on the noted protocol. The average results of the two reference systems are shown in the graph along with actual results that were plotted vs. expected sperm concentrations (Fig. 1).

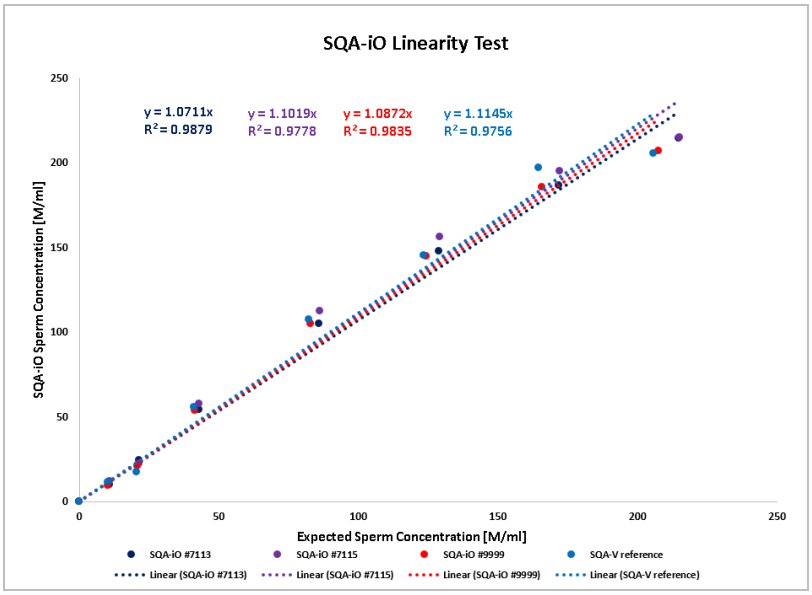

Figure 1. SQA-iO & SQA-V PREDICATE results vs. expected values over device dynamic range

The linearity regression coefficients and percentage recovery established over the SQA-iO device dynamic range are shown in Table 7.

Table 7. Linearity parameters vs. MES claims throughout the measuring range of the SQA-iO device vs. SQA reference PREDICATE.

SQA-iO	Linearity Regression R	MES claim	Linearity Recovery, %	MES claim
#7113	0.994	0.9	110.1	100+/-15%
#7115	0.989	0.9	114.8	100+/-15%
#9999	0.992	0.9	110.3	100+/-15%
SQA-V PREDICATES	0.988	0.9	113.0	100+/-15%

Linearity regression coefficients of all SQA-iO devices and SQA PREDICATES exceed MES claims. The linearity percentage recovery of all tested systems are within the MES claim limits.

Study conclusions

- The SQA-iO device measuring range is similar to the SQA PREDICATE.
- SQA-iO regression coefficients R of linearity trendlines exceed the MES claim of 0.9.
- Linearity percentage recovery vs. expected values are within the MES claim range of 100 +/-15%.

References

- WHO laboratory manual for the examination and processing of human semen - 5th ed., World Health Organization 2010.

5. Definition of Assay Cut-off

The SQA-iO is a point-of care medical diagnostic device (MDD) designed to perform semen analysis. It is not an assay so this type of analysis (trial) is not relevant. The reference values (cut-offs) for semen parameters tested by any method including the SQA-iO device are established and provided to the users by the WHO 5th ed. manual.

References

- WHO laboratory manual for the examination and processing of human semen - 5th ed., World Health Organization 2010.