



SQA-VISION VALIDATION REPORT

Objective

To test the new convenience features of the SQA-Vision vs. the SQA-V and manual semen assessment:

- Swim-up
- Density Gradient
- Longevity
- Vitality
- Morphology Counter
- Scanning Debris/Round Cells
- SQA-Vision Visualization Validation

These parameters were always available to the SQA-V customers, but were tracked or recorded manually on separate pages or forms. The new SQA-Vision system is based on the SQA-V platform (1), but streamlines the assessment and data collection processes by allowing this information to be part of the standard patient record. The objective of this trial is to determine that these semen assessment features perform as designed, and provide the correct results vs. reference method (SQA-V and manual semen assessment where applicable) and vs. manufacturer claims. None of these convenience features include new algorithms for reporting semen values. Additionally, some of the features are not run automatically on the SQA-Vision, but are run manually using the visualization system of the SQA-Vision instead of the microscope. In these cases, the microscope will be considered the GOLD STANDARD for comparison.

Study Design

- Two clinical trials were conducted: at MES Ltd., Israel (169 semen samples of the donors), at the University Hospital of Nantes, France (57 semen samples from patients being evaluated at the andrology unit). In total, 226 human semen samples were tested.
- Types of semen samples/tests run: Fresh, Washed, Swim-up, Density Gradient, Longevity, Vitality, Morphology using the SQA-Vision counter, samples with Debris/Round Cells, Visualization compartment validation.
- All semen samples were collected, split into two aliquots and run according to WHO 5th edition manual (2) standards and per the manufacturer's protocol for comparison:
 - Split into two aliquots each homogeneously mixed semen sample after complete liquefaction
 - If samples do not liquefy completely or are highly viscous, treat with chymotrypsin (QwikCheck™ Liquefaction Kit) and only then split into two aliquots
 - Perform all testing at room temperature (RT)
- **Motility and Progressive Motility Assessment:**
 - Assess one aliquot first under the microscope in duplicate as defined by WHO 5th edition manual, within 1 hour of collection (and directly following sample liquefaction) and in parallel test the second aliquot on the SQA-Vision.
- **Manual concentration :**
 - Assess using an Improved Neubauer chamber following WHO 5th table for comparing duplicate counts
 - Count a minimum of 200 cells, twice as required by the WHO 5th edition manual
 - Use a minimum of 100 µl of raw semen for any dilutions in order to avoid dilution errors
 - Use positive displacement calibrated lab pipettes



- **Manual morphology:**
 - Prepare Papanicolaou stained smears and assess them based on WHO 5th guidelines for normal/abnormal morphology
- **SQA-V:**
 - Run the samples per SQA-V onscreen instructions following the present protocol guidelines (for each sample, run in duplicate using the same capillary)
 - Save results by clicking Import Test in the V-Sperm
- **SQA-Vision:**
 - Run the samples per SQA-Vision onscreen instructions following the present protocol guidelines (for each sample, run in duplicate using the same capillary)
- **SQA-Vision Visualization:**
 - The SQA-Vision Visualization was validated vs. the microscope (Neubauer chamber) and vs. SQA-V Visualization as follows:
 - ✓ Run the same varying concentrations of 20 sperm samples and 8 latex beads samples of varying concentrations on the SQA-Vision Visualization screen (manual mode)
 - ✓ Run the same samples using the Improved Neubauer counting chamber and additionally on the SQA-V Visualization and compare results.

Materials and Methods

Equipment and materials

- SQA-Vision system
- SQA-V System + V-Sperm
- Improved Neubauer chamber
- Phase contrast microscope
- Centrifuge with 15 ml tubes for centrifugation
- Freezing vials
- Egg Yolk Freezing media
- Pasteur pipettes
- Standard lab slides and cover slips 22 X 22 mm
- Fixed coverslip slides
- SQA-V capillaries
- CellVu pre-stained morphology slides
- Papanicolaou reagents
- Eosin reagent for Vitality test (see recipe below)
- Optiprep density gradient medium
- 2.77% and 1.56% NaCl
- Fresh, Washed, Swim-up, Density Gradient and Longevity semen samples
- Lab pipettes + tips, calculators, counters, data collection sheets
- 10 ml cups with caps
- Gloves, Kimwipes
- QwikCheck™ Beads, Test Strips, Liquefaction and Dilution kits

SQA-Vision default settings

- Concentration standard: Neubauer
- Testing criteria: WHO 5th



- Automated opening of video screen for debris/round cells scanning:
Concentration < 15M/ml OR Motility < 40%
- Longevity: 3 tests - initial test is run at 0 point, then two more tests in 2h intervals; all three records are shown in one report
- Manual Morphology set to Normal/Abnormal
- Vitality: Yes
- QC: QwikCheck beads according to the kit labeling (Run 3 levels of QwikCheck beads control daily before testing samples).

Sample inclusion/exclusion criteria

- Highly viscous or incompletely liquefied semen samples treated with the QwikCheck™ Liquefaction kit are included in the trial
- Semen samples with volume < 1 ml are excluded from the trial
- Samples with methodological issues are excluded from the trial (6 samples)

Sample collection and preparation

Fresh Samples:

- Collect Fresh samples according to WHO 5th manual procedure
- Liquefy for up to 1 hour. If not liquefied, treat with 1 vial of QwikCheck Liquefaction Kit (α -chymotrypsin) and test after complete liquefaction (within 10 minutes)
- Mix each sample thoroughly before proceeding
- Mark each sample with a unique sample #, and then split into two aliquots:
 - SQA-Vision and SQA-V: 1-1.5 ml
 - Microscope: remaining sample with minimum of 100 μ l
- Do not dilute the sample before splitting it into aliquots. If diluting, follow the manufacturer's instructions based on the method used
- Run the sample in the Fresh mode of the SQA-Vision and SQA-V

Washed Samples:

- Add 2 ml semen and up to 10 ml Earle's buffer to the centrifuge tube, mix thoroughly
- Centrifuge at 220g for 10 min
- Remove the supernatant
- Re-suspend the pellet with 1 ml Earle's buffer
- Run the sample in the Washed mode of the SQA-Vision and SQA-V

Swim-up Samples:

- Transfer 2 ml of a Fresh semen sample into the test tube.
- Layer 2ml Earle's buffer on the top (do not mix with sample)
- Incline the tube to a 45^o angle
- Incubate the tube for 1 hour at 37^oC
- Collect the upper layer of Earle's buffer (~ 2 ml)
- Run the collected sample in the Swim-up mode of the SQA-Vision and the low volume washed mode of the SQA-V



Density Gradient Samples:

- Prepare the Density Media:
 - 40% OptiPrep: 1.35 ml of stock OptiPrep + 0.65 ml 2.77% NaCl
 - 25% OptiPrep: 0.85 ml of stock OptiPrep + 1.15 ml 1.56% NaCl
- Place up to 1 ml (about half of the semen volume) of 40% Gradient solution into a tube.
- Carefully pour up to 1 ml (about half of semen volume) of 25% Gradient solution, along the wall of the tube in order to create two layers.
- Mix the semen sample and add the semen gently along the tube wall to create a third layer. If the semen volume is more than 3ml distribute the samples into two tubes.
- Centrifuge at 300g (1350 rpm) for 20 minutes.
- Collect the pellet into a new tube.
- Re-suspend the pellet with 1 ml Earle's buffer.
- Run the collected sample in the Density Gradient mode of SQA-Vision and low volume washed mode of the SQA-V.

Longevity Test:

- Fill the capillary with fresh sample, run the initial test in the SQA-Vision Longevity mode and in the SQA-V
- Expel the sample from the capillary into a 10-ml vial and incubate for 2 hours
- After incubation, re-run the test in the SQA-Vision Longevity mode and manually for motility and progressive motility
- Save results
- Repeat the same procedure after 4 hours

Running Samples

Parameters for comparison:

- The SQA-Vision semi-automated results obtained by running fresh, washed and longevity samples with moderate/high level of debris/round cells or Concentration or MSC beyond automated dynamic range (<2M/ml) vs. the microscope (manual): concentration, motility, progressive motility and morphology
- The SQA-Vision fully automated mode (fresh, washed and longevity samples with a low level of debris/round cells and Concentration and MSC \geq 2M/ml) vs. the SQA-V: Concentration, motility, progressive motility and morphology
- The SQA-Vision fully automated mode for swim-up and density gradient samples vs. the SQA-V: Motile Sperm Concentration (MSC) and Progressively Motile Sperm Concentration (PMSC) reported by both systems
- The SQA-Vision CellVu™ Morphology to microscope (manual) Papanicolaou (PAP) Morphology
- The SQA-Vision Vitality to manual (microscope) Vitality

SQA-Vision and SQA-V sample testing:

- Turn on the SQA-Vision and the SQA-V.
- Activate the Vision HOME screen and V-Sperm.
- Set the SQA-V and SQA-Vision default settings according to the setting instructions listed above.



- Run 3 levels of QwikCheck™ Beads control material before testing samples.
- Prepare the Morphology slide in advance: CellVu pre-stained slide, 5 µl semen and 22mm x 22mm coverslip pressed by finger to make a cell monolayer.
- Prepare in advance Vitality slide:

Reagent Preparation

1. NaCl, 0.9% (w/v): dissolve 0.9 g of NaCl in 100 ml purified water
2. Eosin Y, 0.5% (w/v): dissolve 0.5 g of eosin Y (color index 45380) in 100 ml of 0.9% NaCl

Procedure

1. Mix the semen sample well
 2. Remove an aliquot of 5 µl of semen and combine with 5 µl of eosin solution on a microscope slide
 3. Mix with a pipette tip, swirling the sample on the slide
 4. Cover with a 22 mm x 22 mm coverslip and leave at least for 30 seconds
- Enter the patient/sample data.
 - Assess the pH and WBC using QwikCheck™ Test Strips and enter their values where appropriate.
 - Follow the onscreen instructions of each system and run the test.
 - If the results fall below the cut-offs for low quality semen (Concentration or MSC <2M/ml), the SQA-Vision will open the manual sample assessment screen automatically.
 - The SQA-V automatically displays a limited report in this case (Concentration <2M/ml, etc.); Then the SQA-Vision manual results will be compared to the microscope manual data.
 - To use the Vision Manual Assessment Screen: Fill a fixed coverslip slide with semen sample per product and SQA-Vision onscreen instructions. View the slide in the visualization compartment of the SQA-Vision and count the motile and immotile sperm cells manually (at least 200 spermatozoa in ~ 10-20 fields)
 - Enter the manual data in the fields provided and obtain a report.
 - Run a second testing cycle using the same testing capillary and sample.
 - Run the Morphology (CellVu) and Vitality (eosin) tests.
 - Insert the Morphology slide into the SQA-Vision visualization compartment and assess normal and abnormal spermatozoa using the counter (at least 200 spermatozoa in approx. 10-20 fields of view).
 - Insert the Vitality slide into the SQA-Vision visualization compartment and assess using the counter.
 - Count live (non-stained) and dead (stained) spermatozoa (at least 200 spermatozoa twice in approx. 10-20 fields of view) at zoom out.
 - In parallel, examine the slides under the microscope.
 - Save the data.

Manual Motility:

- Assess motility within one hour of collection, immediately after full liquefaction of the sample.
- Run all tests in the SQA-Vision, SQA-V and the microscope in parallel or within 2 minutes of each other to eliminate the effect of time difference.

Assessing motility under the microscope

1. Count each sample in duplicate
2. Mix the semen sample well



3. Remove an aliquot of semen immediately after mixing, allowing no time for the spermatozoa to settle
4. For each replicate, prepare a wet preparation approximately 20 μm deep
5. Prepare standard slides according to WHO 5th manual (p. 21): Load 10 μL of semen onto a standard microscope slide and cover with 22mm X 22mm coverslip providing 20-micron depth
6. Wait for the sample to stop drifting (within 60 seconds)
7. Examine the slide under phase-contrast optics at x200 or x400 magnification
8. Assess 200 spermatozoa per replicate to determine the percentage of different motile categories
9. Per WHO 5th ed. manual (p. 22), the motility of each spermatozoon is graded as follows:
 - Progressive motility (PR): spermatozoa moving actively, either linearly or in a large circle, regardless of speed
 - Non-progressive motility (NP): all other patterns of motility with an absence of progression, e.g., swimming in small circles, the flagella force hardly displacing the head, or when only a flagellar beat can be observed
 - Immotility (IM): no movement
10. Compare the replicate values to check if they are acceptably close per WHO 5th table 2.1. If so, average results; if not, prepare and assess new samples
11. Record Motility values of each sample in the Assessment Form

Manual Sperm Concentration:

1. Use the Neubauer counting chamber for the assessment of concentration following the WHO 5th table (table 2.3 below)
2. Count a minimum of 200 cells twice
3. To compare duplicate count accuracy use WHO 5th table 2.4 (below)
4. Record the Sperm Concentration values for each sample in the Assessment Form provided

Manual Morphology:

Follow the steps outlined in the WHO 5th manual (Section 2.13 Sperm morphology, p. 56) recommended for testing morphology:

1. Prepare a minimum of two smears of fresh semen (10 μl) for duplicate measurements
2. Stain using Papanicolaou method
3. Mount the slide with a coverslip if the slide is to be kept for a long time

Morphology Counting

1. Examine the slide under oil immersion with bright field optics at x1000 magnification
2. Morphological evaluation should be performed in several systematically selected areas of the slide
3. Count all spermatozoa per WHO 5th criteria, noting only the # Normal and # Abnormal forms (not the differential of defects) in order to establish the % normal forms. If appropriate, count differential morphology as well
4. Compare replicate values to WHO 5th table 2.1 to determine acceptability; If not, re-read the slides
5. Record the Morphology values of each sample in the Assessment Form provided



Table 2.1 Acceptable differences between two percentages for a given average, determined from replicate counts of 200 spermatozoa (total 400 counted)

Average (%)	Acceptable Difference*	Average (%)	Acceptable Difference*
0	1	66-76	9
1	2	77-83	8
2	3	84-88	7
3-4	4	89-92	6
5-7	5	93-95	5
8-11	6	96-97	4
12-16	7	98	3
17-23	8	99	2
24-34	9	100	1
35-65	10		

*Based on the rounded 95% confidence interval.

Table 2.3 Semen dilutions required, how to make them, which chambers to use and potential areas to assess

Spermatozoa per x400 field	Spermatozoa per x200 field	Dilution required	Semen (µl)	Fixative (µl)	Chamber	Area to be assessed
>101	>404	1:20 (1 + 19)	50	950	Improved Neubauer	Grids 5, 4, 6
16-100	64-400	1:5 (1 + 4)	50	200	Improved Neubauer	Grids 5, 4, 6
2-15	8-60	1:2 (1 + 1)	50	50	Improved Neubauer	Grids 5, 4, 6
<2	<8	1:2 (1 + 1)	50	50	Improved Neubauer or large-volume	All 9 grids Entire slide

Table 2.4 Acceptable differences between two replicate counts for a given sum

Sum	Acceptable Difference*	Sum	Acceptable Difference*
144-156	24	329-346	36
157-169	25	347-366	37
170-182	26	367-385	38
183-196	27	386-406	39
197-211	28	407-426	40
212-226	29	427-448	41
227-242	30	449-470	42
243-258	31	471-492	43
259-274	32	493-515	44
275-292	33	516-538	45
293-309	34	539-562	46
310-328	35	563-587	47

*Based on the rounded 95% confidence interval.



Statistics and Acceptance Criteria

Statistical analysis was performed using the MedCalc and Excel programs:

- Correlation
- Sensitivity (an ability to detect abnormal cases vs. reference method)
- Specificity (an ability to detect normal cases vs. reference method)
- Precision: CV (coefficient of variation)

Acceptance Criteria is based on the SQA-Vision product performance claims (applicable to any sample type/test results including Longevity presented in the study):

- Correlation:
 - Sperm Concentration: ≥ 0.90
 - Total Motile (PR + NP): ≥ 0.80 (in Longevity, each step is compared vs. reference method as a separate test)
 - Progressive Motility (PR): ≥ 0.80 (same as above)
 - MSC: ≥ 0.85
 - PMSC: ≥ 0.80
 - Vitality: ≥ 0.90
- Sensitivity:
 - Sperm Concentration: $\geq 90\%$
 - Total Motile (PR + NP): $\geq 85\%$
 - Progressive Motility (PR): $\geq 85\%$
 - Normal Forms: $\geq 80\%$
 - MSC: $\geq 85\%$
 - PMSC: $\geq 85\%$
 - Vitality: $\geq 90\%$
- Specificity:
 - Sperm Concentration: $\geq 85\%$
 - Total Motile (PR+NP): $\geq 80\%$
 - Progressive Motility (PR): $\geq 80\%$
 - Normal Forms: $\geq 90\%$
 - MSC: $\geq 80\%$
 - PMSC: $\geq 80\%$
 - Vitality: $\geq 90\%$
- SQA-Vision coefficients of variation (CV) for Concentration, Total Motile and Progressive Motility parameters $<10\%$
- SQA-Vision Visualization: Correlation to Neubauer ≥ 0.9

Results

Sperm Concentration

The Sperm Concentration results from a variety of semen sample types run on the SQA-Vision were statistically compared to the SQA-V and to manual microscope results (for semi-automated or the manual mode of the SQA-Vision). The results are summarized in the Table 1 below:

Table 1: SQA-Vision Sperm Concentration							
TP	TN	FP	FN	Sensitivity ^{1,2} (%)	Specificity ^{1,2} (%)	Correlation (r)	SQA-Vision Intra-device CV (%)
MES Ltd., Israel (n = 169)							
30	139	0	0	100.0	100.0	0.98	4.5
University Hospital of Nantes, France (n = 57)							
25	31	0	1	96.2	100.0	0.95	4.1

¹Sperm Concentration reference value used for calculation sensitivity and specificity per WHO 5th ed. manual is 15 M/ml

²Sensitivity = TP / (TP + FN) * 100; Specificity = TN / (TN + FP) * 100

Notes:

TP - True Positive (correctly classified as positive - presence of disease)

TN - True Negative (correctly classified as negative - absence of disease)

FP - False Positive (not correctly classified as positive - absence of disease)

FN - False Negative (not correctly classified as negative - presence of disease)

The correlation coefficients of the SQA-Vision Sperm Concentration to the SQA-V + manual results are high (0.98; 0.95). They exceed the acceptance criteria. This high correlation shows strong agreement between the methods. The tight relationship between Sperm Concentration reported by the SQA-Vision and SQA-V + manual methods is demonstrated graphically in Figure 1:

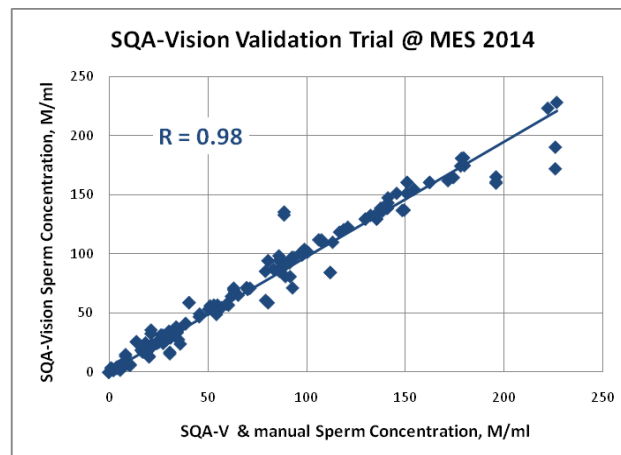


Figure 1. SQA-Vision vs. SQA-V and manual Sperm Concentration

The SQA-Vision Sperm Concentration Sensitivity and Specificity obtained in the different trials greatly exceeds the acceptance cutoff of $\geq 90\%$ and 85% correspondingly. The SQA-Vision also demonstrated excellent precision for assessing Sperm Concentration with low coefficients of variation of 4.5% and 4.1%, which meet the acceptance requirements.

Total Motility (PR + NP)

The Total Motility sperm results from a variety of semen sample types run on the SQA-Vision were statistically compared to the SQA-V and to manual microscope results (for semi-automated or the manual mode of the SQA-Vision). The results are summarized in the Table 2 below:

Table 2: SQA-Vision Total Motility							
TP	TN	FP	FN	Sensitivity ^{1,2} (%)	Specificity ^{1,2} (%)	Correlation (r)	SQA-Vision Intra-device CV (%)
MES Ltd., Israel (n = 178)							
59	110	4	5	92.2	96.5	0.92	6.2
University Hospital of Nantes, France (n = 54)							
11	36	6	1	91.7	85.7	0.85	5.0

¹Total Motility reference value used for calculation sensitivity and specificity per WHO 5th ed. manual is 40%

²Sensitivity = TP / (TP + FN) * 100; Specificity = TN / (TN + FP) * 100

Notes:

TP - True Positive (correctly classified as positive - presence of disease)

TN - True Negative (correctly classified as negative - absence of disease)

FP - False Positive (not correctly classified as positive - absence of disease)

FN - False Negative (not correctly classified as negative - presence of disease)

The correlation coefficients of the SQA-Vision Total Motility to the SQA-V + manual results are high (0.92; 0.85) and these results equal or exceed the acceptance criteria. This high correlation shows strong agreement between the methods. This tight relationship between Total Motility reported by the SQA-Vision and the SQA-V + manual methods is demonstrated graphically in Figure 2:

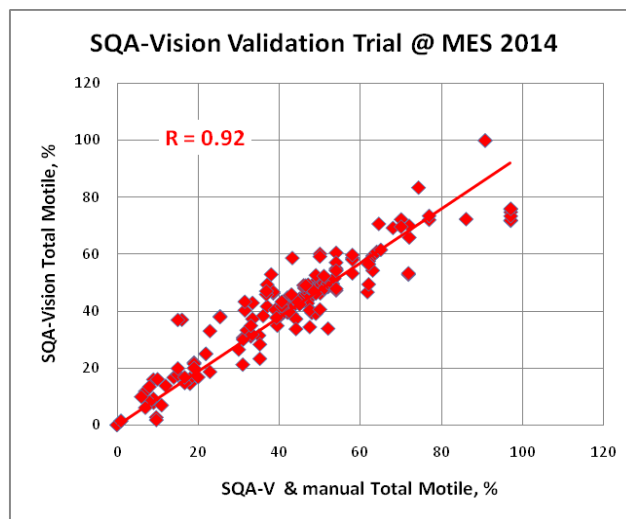


Figure 2. SQA-Vision vs. SQA-V and manual Total Motility

The SQA-Vision Total Motility Sensitivity and Specificity are high and exceed the acceptance cutoff. The SQA-Vision also demonstrated excellent precision when measuring Total Motile sperm demonstrated by low coefficients of variation of (6.2%; 5.0%) which meet the acceptance requirements.

Progressive Motility (PR)

The Progressive Motility results from a variety of semen sample types run on the SQA-Vision were statistically compared to the SQA-V and to manual microscope results (for semi-automated or manual mode of the SQA-Vision). The results are summarized in the Table 3:

Table 3: SQA-Vision Progressive Motility							
TP	TN	FP	FN	Sensitivity ^{1,2} (%)	Specificity ^{1,2} (%)	Correlation (r)	SQA-Vision Intra-device CV (%)
MES Ltd., Israel (n = 161)							
59	94	3	5	92.2	96.9	0.91	5.8
University Hospital of Nantes, France (n = 36)							
14	19	2	1	93.3	90.5	0.85	4.7

¹Progressive Motility reference value used for calculation sensitivity and specificity per WHO 5th ed. manual is 32%

²Sensitivity = TP / (TP + FN) * 100; Specificity = TN / (TN + FP) * 100

Notes:

TP - True Positive (correctly classified as positive - presence of disease)

TN - True Negative (correctly classified as negative - absence of disease)

FP - False Positive (not correctly classified as positive - absence of disease)

FN - False Negative (not correctly classified as negative - presence of disease)

The correlation coefficients of the SQA-Vision Progressive Motility results vs. the SQA-V + manual results are high (0.91; 0.85) and are equal to or exceed the acceptance criteria. This high correlation shows strong agreement between the methods. This tight relationship between Progressive Motility reported by the SQA-Vision and the SQA-V + manual method is demonstrated graphically in Figure 3:

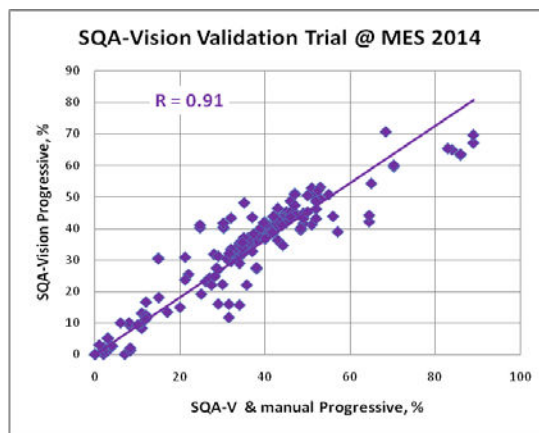


Figure 3. SQA-Vision vs. SQA-V and manual Progressive Motility

The SQA-Vision Progressive Motility Sensitivity and Specificity are high and exceed the acceptance cutoff. The data shows that only a few samples were categorized differently between the two systems. This can be due to the subjectivity of the manual Progressive Motility assessment. The SQA-Vision also demonstrated excellent precision for assessing Progressively Motile sperm as demonstrated by low coefficients of variation which meet the acceptance requirements.

Samples with Debris and/or Round Cells

An ejaculate contains cells other than spermatozoa, some of which may be clinically relevant. These include epithelial cells from the genitourinary tract, as well as leukocytes and immature germ cells, the latter two collectively referred to as “round cells” (3). Besides these elements, the ejaculate may contain debris (particles of broken cells). These inclusions are interfering factors for both manual and automated semen assessment (2).

The results of running semen samples containing debris and/or round cells on the SQA-Vision as compared to running these same samples manually under the microscope are summarized in Table 4 below.

Table 4: SQA-Vision Samples with Debris and /or Round Cells							
Semen Parameters	TP	TN	FP	FN	Sensitivity ^{1,2} (%)	Specificity ^{1,2} (%)	SQA-Vision Intra-device CV (%)
MES Ltd., Israel							
Sperm Concentration (x10 ⁶ /mL) n = 38	8	30	0	0	100.0	100.0	3.1
Total Motile PR + NP (%) n = 38	14	20	3	1	93.3	87.0	8.5
Progressive PR (%) n = 36	17	13	3	3	85.0	81.3	8.2
University Hospital of Nantes, France							
Sperm Concentration (x10 ⁶ /mL) n = 17	4	13	0	0	100.0	100.0	4.5
Total Motile PR + NP (%) n = 18	2	13	3	0	100.0	81.3	5.4
Progressive PR (%) n = 16	6	8	1	1	85.7	88.9	6.3

¹The reference values for semen parameters used for calculation sensitivity and specificity were per WHO 5th ed. manual

²Sensitivity = TP / (TP + FN) * 100; Specificity = TN / (TN + FP) * 100

Notes:

TP - True Positive (correctly classified as positive - presence of disease)

TN - True Negative (correctly classified as negative - absence of disease)

FP - False Positive (not correctly classified as positive - absence of disease)

FN - False Negative (not correctly classified as negative - presence of disease)

The results demonstrate that the SQA-Vision Sensitivity and Specificity for Sperm Concentration, Total Motility and Progressive Motility are very high which equals or exceeds the acceptance criteria. The SQA-Vision also demonstrated very good precision as shown by coefficients of variation varying from 3.1% to 8.5%, which meet the pass criteria.

Swim-up and Density Gradient Samples

Swim-up and Density Gradient procedures are used when spermatozoa need to be separated from the seminal plasma. The separation of human spermatozoa from the seminal plasma to yield a final preparation containing a high percentage of morphologically normal and motile cells, free from debris, non-germ cells and dead spermatozoa, is important for clinical practice (2).

The choice of sperm preparation technique is dictated by the nature of the semen sample (4). For example, a direct swim-up technique is often used when the semen samples are considered to be largely normal, whereas in cases of severe oligozoospermia, teratozoospermia or asthenozoospermia, density gradients are usually preferred

because of the greater total number of motile spermatozoa recovered. Density-gradients can also be altered to optimize the handling of specific properties of individual samples (2).

The Swim-up and Density Gradient modes of the SQA-Vision and the Washed low volume mode of the SQA-V require a minimum of 20 μL of semen volume (only the front section of the testing capillary is filled). In these cases, motility-related parameters such as Motile and Progressively Motile Sperm Concentration (MSC and PMSC correspondingly) are reported. The results of running Swim-up and Density Gradient samples on the SQA-Vision compared to the SQA-V are summarized in Table 5:

Table 5: SQA-Vision Swim-up and Density Gradient Samples								
Semen Parameters	TP	TN	FP	FN	Sensitivity ^{1,2} (%)	Specificity ^{1,2} (%)	Correlation (r)	SQA-Vision Intra-device CV (%)
MES Ltd., Israel								
MSC ($\times 10^6/\text{mL}$) n = 40	22	18	0	0	100.0	100.0	0.99	8.9
PMSC ($\times 10^6/\text{mL}$) n = 40	24	16	0	0	100.0	100.0	0.99	7.5

¹The MSC and PMSC reference values used for calculation sensitivity and specificity are 6.0 and 5.0 ($\times 10^6/\text{mL}$) correspondingly

²Sensitivity = $\text{TP} / (\text{TP} + \text{FN}) * 100$; Specificity = $\text{TN} / (\text{TN} + \text{FP}) * 100$

Notes:

TP - True Positive (correctly classified as positive - presence of disease)

TN - True Negative (correctly classified as negative - absence of disease)

FP - False Positive (not correctly classified as positive - absence of disease)

FN - False Negative (not correctly classified as negative - presence of disease)

The MSC and PMSC Sensitivity and Specificity for both systems were 100%. The correlation coefficients of these parameters obtained using the SQA-Vision system vs. SQA-V are 0.99 (both) which exceeds the acceptance criteria. The high sensitivity, specificity and correlations demonstrate a very high level of agreement between the methods. This tight relationship between the semen parameters reported by the SQA-Vision and the SQA-V is demonstrated graphically in Figures 4 and 5 below.

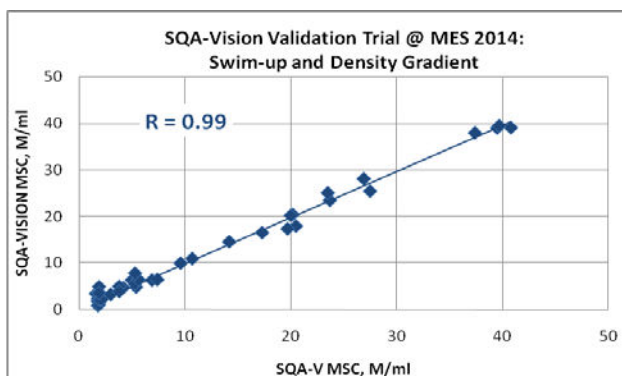


Figure 4. SQA-Vision vs. SQA-V MSC

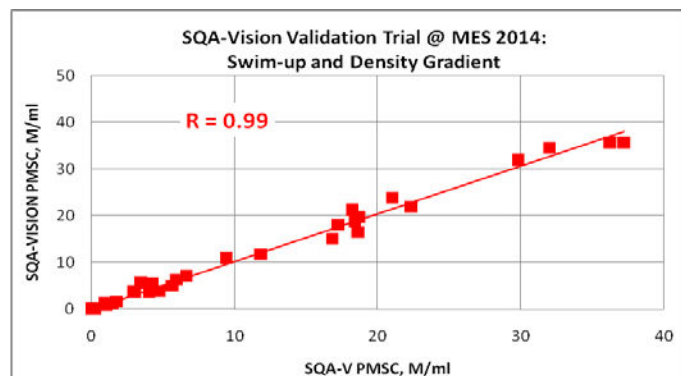


Figure 5. SQA-Vision vs. SQA-V PMSC

The SQA-Vision also demonstrated good precision when assessing MSC and PMSC as evidenced by coefficients of variation below 10% which meet the acceptance requirements.

Longevity

Longevity testing is a requirement in many laboratories. The length of time sperm are able to remain viable after ejaculation may be of concern in some cases. The sperm longevity test assesses percent Total Motile and Progressive sperm at given time intervals.

The results of running longevity tests on the SQA-Vision compared to the SQA-V and the manual method (for comparing the semi-automated or manual mode of the SQA-Vision) are summarized in Table 6 below:

Table 6: Longevity							
Semen Parameters	TP	TN	FP	FN	Sensitivity ^{1,2} (%)	Specificity ^{1,2} (%)	Correlation (r)
MES Ltd., Israel							
Total Motile PR + NP (%) n = 30	6	24	0	0	100.0	100.0	0.84
Progressive PR (%) n = 30	7	21	1	1	87.5	95.5	0.84
University Hospital of Nantes, France							
Total Motile PR + NP (%) n = 20	8	10	2	0	100.0	83.3	0.92
Progressive PR (%) n = 16	8	8	0	0	100.0	100.0	0.93

¹Reference values for semen parameters used for calculating sensitivity/specificity are per WHO 5th ed. manual

²Sensitivity = TP / (TP + FN) * 100; Specificity = TN / (TN + FP) * 100

Notes:

TP - True Positive (correctly classified as positive - presence of disease)

TN - True Negative (correctly classified as negative - absence of disease)

FP - False Positive (not correctly classified as positive - absence of disease)

FN - False Negative (not correctly classified as negative - presence of disease)

The results demonstrate high correlation coefficients for the SQA-Vision percent Total Motile and Progressive Motility parameters to the SQA-V + manual results; varying from 0.84 to 0.93 which exceeds the acceptance criteria. This high correlation shows good agreement between the methods. The relationship between Total Motile and Progressive Motility parameters reported by the SQA-Vision and SQA-V + manual method in the Longevity samples is demonstrated graphically in Figures 6 and 7:

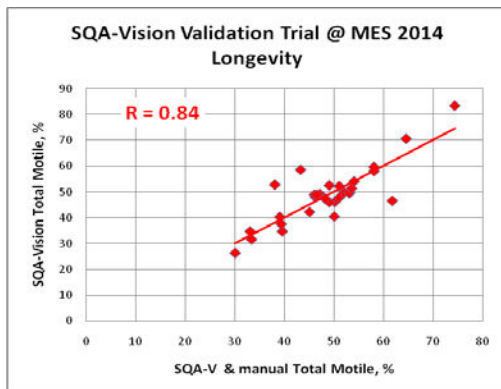


Fig. 6. SQA-Vision vs. SQA-V & manual Total Motile

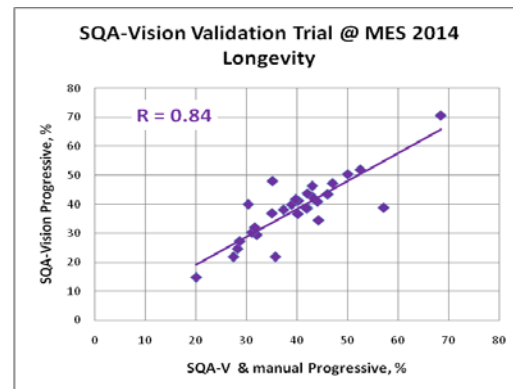


Fig. 7. SQA-Vision vs. SQA-V & manual Progressive

The sensitivity of Total Motile and Progressive Motility parameters assessed by the SQA-Vision for Longevity testing is high varying from 87.5% to 100% which exceed the acceptance cutoff. The specificity of these parameters for the Longevity tests is also high varying from 83.3% to 100% which is greater than the acceptance criteria.

Vitality

Sperm Vitality, as estimated by assessing the membrane integrity of the cells, may be determined routinely on all samples, but is especially important for samples with less than about 40% progressively motile spermatozoa. This test can provide a check on the motility evaluation, since the percentage of dead cells should not exceed (within sampling error) the percentage of immotile spermatozoa. The percentage of viable cells normally exceeds that of motile cells. The percentage of live spermatozoa is assessed by identifying those with an intact cell membrane, using dye exclusion or hypotonic swelling. The principle of the dye exclusion method is that damaged plasma membranes, such as those found in non-vital (dead) cells, allow entry of membrane-impermeant stains (2).

The results of assessing eosin-stained semen for Vitality using the SQA-Vision visualization compartment compared to the microscope manual method are summarized in Table 7 below:

Table 7: Vitality (% Live)						
TP	TN	FP	FN	Sensitivity ^{1,2} (%)	Specificity ^{1,2} (%)	Correlation (r)
MES Ltd., Israel (n = 49)						
10	37	2	0	100.0	94.9	0.91

¹Vitality reference value per WHO 5th ed. manual is 58%.

²Sensitivity = TP / (TP + FN) * 100; Specificity = TN / (TN + FP) * 100

Notes:

TP - True Positive (correctly classified as positive - presence of disease)

TN - True Negative (correctly classified as negative - absence of disease)

FP - False Positive (not correctly classified as positive - absence of disease)

FN - False Negative (not correctly classified as negative - presence of disease)

The correlation coefficient of the SQA-Vision Vitality to the manual results is 0.91 which exceeds the acceptance criteria. This high correlation shows good agreement between the methods. The tight relationship between Vitality reported by the SQA-Vision and the manual method is demonstrated graphically in Figure 8:

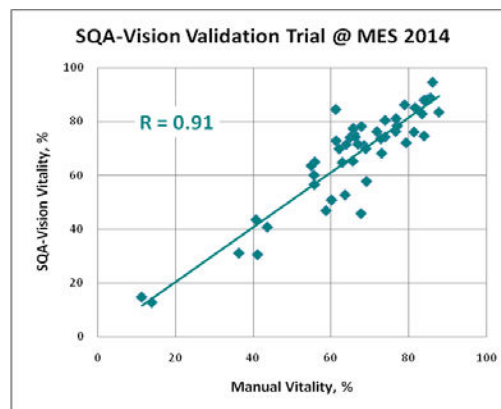


Figure 8. SQA-Vision vs. manual Vitality

The sensitivity and specificity of the SQA-Vision to assess Vitality vs. the manual results is very high: 100% and 94.9% respectively which exceeds the acceptance cutoff.



Morphology

Summarized in Table 8 below are the results of Morphologically Normal Forms obtained by:

- SQA-Vision automatically
- The use of CellVu pre-stained slides counted in the SQA-Vision visualization compartment
- Manual assessment of Papanicolaou (PAP) smears

Table 8: Normal Forms (Morphology)						
Semen Parameters	TP	TN	FP	FN	Sensitivity ^{1,2} (%)	Specificity ^{1,2} (%)
MES Ltd., Israel						
SQA-Vision auto vs. CellVu (n = 45)	8	32	3	2	80.0	91.4
SQA-Vision CellVu vs. Manual PAP (n = 50)	8	37	3	2	80.0	92.5
University Hospital of Nantes, France						
SQA-Vision auto vs. Manual PAP (n = 43)	10	30	1	2	83.3	96.8

¹Normal Forms (Morphology) reference value per WHO 5th ed. manual is 4%.

²Sensitivity = TP / (TP + FN) * 100; Specificity = TN / (TN + FP) * 100

Notes:

TP - True Positive (correctly classified as positive - presence of disease)

TN - True Negative (correctly classified as negative - absence of disease)

FP - False Positive (not correctly classified as positive - absence of disease)

FN - False Negative (not correctly classified as negative - presence of disease)

The sensitivity and specificity of the SQA-Vision normal morphology assessed automatically vs. the SQA-Vision CellVu data is: 80.0% and 91.4% respectively, which exceed the acceptance cutoff. A comparison of the SQA-Vision CellVu morphology with the manual PAP data resulted in sensitivity and specificity of 80.0% and 92.5% correspondingly which also exceeds the acceptance criteria. The SQA-Vision automated percent normal forms compared to manual PAP data showed sensitivity of 83.3% and specificity of 96.8% (both values are higher than the acceptance criteria). Based on this data it is possible to conclude that all three methods are in agreement.

SQA-Vision Visualization Screen Validation

The SQA-Vision Visualization Screen was validated by counting varying concentrations of spermatozoa (20 samples) and latex beads (8 samples) using:

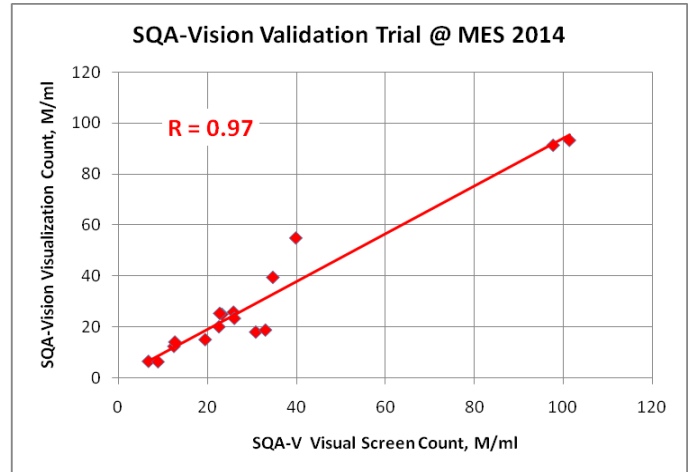
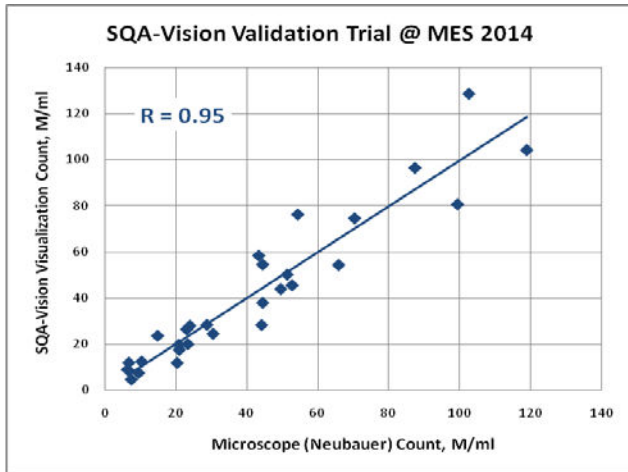
- SQA-Vision Visualization
- Improved Neubauer counting chamber

Additionally, the SQA-Vision Visualization was validated vs. the SQA-V video screen by counting 8 sperm samples and 8 samples of latex beads. The results are summarized in Table 9 below:

Table 9: SQA-Vision Visualization Validation					
Parameter	SQA-Vision Visualization Count		Manual Count		Correlation (r)
	Average	SD	Average	SD	
MES Ltd., Israel					
Count vs. Neubauer (x10 ⁶ /mL) n = 28	42.1	32.3	42.1	31.0	0.95
Count vs. SQA-V visual screen (x10 ⁶ /mL) n = 16	30.7	26.9	32.4	27.8	0.97

It is seen that the average values obtained by manually counting sperm cells and latex beads using the SQA-Vision Visualization System and the Improved Neubauer counting chamber are similar and the standard deviations (SD) are very close. A similar relationship is demonstrated when comparing the results of counting sperm cells and

latex beads using the SQA-Vision vs. SQA-V visualization screen. This indicates that there are no systematic discrepancies between the methods which were compared. The data set also demonstrates a high level of correlation which is shown graphically below:



Conclusions

- Based on results of the multicenter trials, the SQA-Vision demonstrated that all assessed parameters passed the acceptance criteria.
- A high level of correlation between the SQA-Vision and SQA-V automated + manual results (used as the standard when appropriate) demonstrated agreement between these methods.
- The Sensitivity and Specificity levels for all assessed parameters were higher than the acceptance criteria.
- The SQA-Vision demonstrated a high level of precision (all CVs are below 10%).
- The SQA-Vision Visualization can be used for manual counting as an accessory tool.
- The overall conclusion is that the SQA-Vision system successfully passed clinical validation showing substantial equivalence with the SQA-V system.

References

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