

SQA-V *Gold*

USER GUIDE

V e r s i o n 2 . 6 0 I - B u t t o n
W H O 5 t h

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Specifications

SQA-V Gold
Version 2.60

SECTION 1: System Specifications and Requirements

Dimensions: 32 X 30 X 24 cm
Weight: 7 Kg
AC power supply: 100-240 VAC, 50-60 Hz, 20 VA

Archive Capacity

- 500 test records / 750 QC records

Display(s)

- Operational backlight LCD (16 lines x 40 characters)
- Video backlight LCD (8 x 10 cm)

Factory Default Settings

SYSTEM:

Date format: DD/MM/YY

Time/Date: Manufacturer's local time/date

Morphology: WHO 5th

Chamber standard: 2

Printing Options: Automatic

CONTROLS:

Control Media: Latex Beads, Stabilized Sperm CAP or MES (Lot #, Target Values, +/- Ranges set up by user)

Front Panel

- Displays: LCD video display and controls, LCD operational display.
- Testing: Measurement compartment, Visualization compartment.
- Other: Multi-button keypad, I-Button port, Focus knob, Built-in printer.

Keypad

- **Operational keys:** ON/OFF, TEST, PRINT, SERVICE, ARCHIVE (now disabled), DELETE, ENTER, four cursor buttons, ESC, ten numeric buttons (0-9).
- **Video control keys:** ZOOM IN/OUT, ILLUMINATION HIGH/LOW, and MONITOR ON/OFF.

Measurement Compartment

- **Sources of radiant energy** - two LEDs for motility and spectrophotometry channels.
- **Detector system** - two photo detectors - Motility and Optical Density.

Operating System

- **Analysis Time:** Normal Test-75 seconds; Low Quality-2 additional minutes; Postvasectomy - 5 minutes.
- **Software:** Resides on flash memory and drives all man-machine interface functions, runs algorithms for test measurements, and operates visual and automated screens. System can be upgraded from a PC CD-ROM.
- **Motility channel input signal:** Analog, up to 5V.
- **Spectrophotometer channel input signal:** Modulated(kHz)analog, up to 5V.

Printer

- Built-in, Dot Matrix with ribbon cassette (Citizen).
- Non-thermostatic narrow paper with 20 characters per line (Citizen).

Rear Panel

- Power connector w/fuse-holder (fuse 250V,1A), Video connector,RS232 outlet.

Requirements

Visualization Compartment

- White LED illumination system
- CCD, 330 TV lines
- Objective: Standard, x20
- Signal Output: PAL standard
- Zoom system for smooth magnification transition between x300 and x500
- Focus regulator

Maintenance Schedule

- **Daily:** Clean measurement compartment daily when running samples and after every 10-15 tests and/or for ANY spillage. Follow manufacturer's cleaning instructions using manufacturer cleaning kit. (Refer to the appendix section "Cleaning the Capillary/Slide Compartments" in this User Guide). **ONLY use the Manufacturers cleaning kit and cleaning brush or damage will occur to the SQA-V film and the system will not operate!**

Manufacturer Recommendations

- Operate the SQA-V 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.
- When running Postvasectomy tests do not interrupt test cycle nor interfere with system or testing capillary in any way – this test is highly sensitive to any motion and requires complete stability of the system during the 5 minute testing cycle.
- Variations in ambient temperature can affect semen samples. **It is essential that semen samples are not heated** for testing. The SQA-V is calibrated to conduct tests at room temperature: 20-25°C (68-77°F).
- Semen is considered a biologically hazardous material and is subject to individual laboratory protocols for handling such materials and at a minimum:
 - Laboratory coat, mask and gloves for operating personnel protection.
 - Samples handling and waste disposal in specially marked hazardous waste containers.
 - Only personnel trained to work with biologically hazardous materials such as semen should be testing and handling semen.

Operating Temperature

- Maximum operational humidity is 80% for temperatures of up to 31°C with decreasing linearly to 50% at 38°C.
- Operates in a wide range of ambient temperatures (15-38°C) however the system is calibrated to measure semen samples at room temperature: 20-25°C (68-77°F). Note: Extreme ambient temperature may impact the accuracy of motility test results because of the known effect of temperature on human semen.

Operational Environmental conditions:

- System is intended for indoor use at a maximum altitude of 2000m, mains supply fluctuations ±10%, Overvoltage Category II, Pollution Degree II.

PC / Hardware Requirements

Minimum requirements for V-Sperm software:

- **PC:** Intel Core 15 M520 2.4GHz or equivalent
- **RAM:** 4GB
- **Video card:** 3D to support high resolutions 16:10 – 1440X900
- **Video color:** At least 16 bit (65,535)
- **CD ROM drive**
- **300GB free hard disk space** for image capturing (approx. 3000 clips)
- **Monitor Screen:** Color, Wide screen – should support resolution 16:10 or 16:9 (1440X900)
- **Operating system compatibility:** Windows XP and 7; Excel/Word (required for V-Sperm GOLD)
- **Communication Ports:** Two FREE native RS232 ports (one for data transfer and one for LIS); two USB ports
- **EXCEL and WORD required for export function and printing test reports**

Quality Control

- **Internal:** Electronic Self-Test and Auto-Calibration. Runs automatically upon start-up. Reference values are verified prior to each test.
- **External:** Run daily prior to testing or per laboratory protocol. Runs assayed latex bead control: "**QwikCheck™-beads**" (product of Medical Electronic Systems) for concentration and negative control for motility/concentration OR non-assayed: Latex beads or stabilized sperm CAP or MES for concentration.

Sample Testing

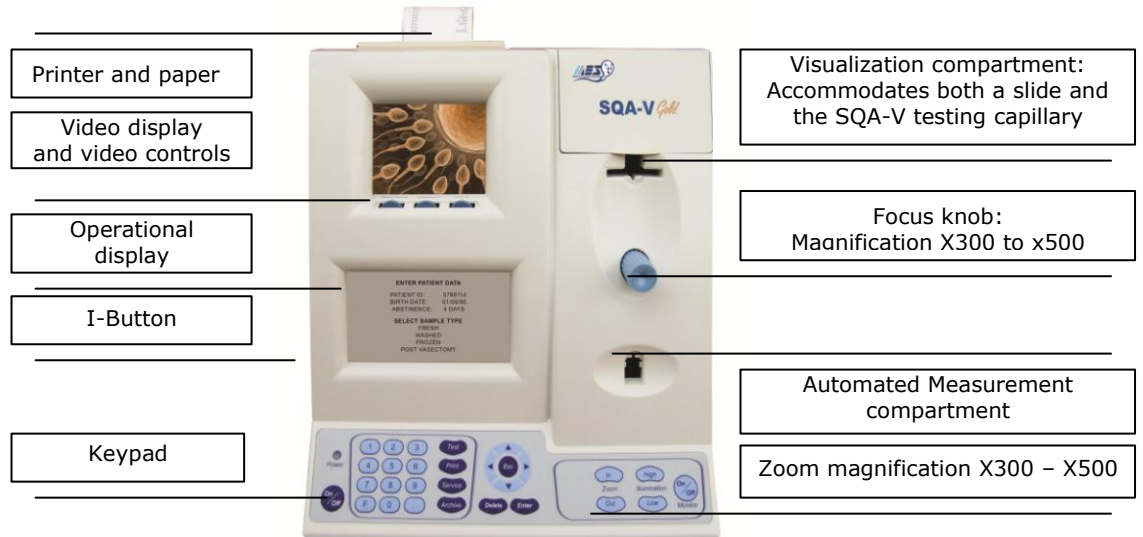
- **Sample Testing Temperature:** Calibrated for room temperature only. Motility results will be impacted by heating the specimen.
- **System calibrated to test Human semen and specified Control samples only.** Not for use with animal semen.
- **SQA-V measurement capillary:** Disposable, plastic, testing capillary. Requires 500 µl of sample for normal volume testing, 20 µl for low volume testing, 300 µl for diluted mode. Use only manufacturers' certified testing capillaries in the automated and visualization system.
- **Slide adaptor:** Supplied with the SQA-V. Must be used with a standard laboratory slide and 22 x 22 mm cover-slip for accurate test results.

Software Required

- **V-Sperm Gold 3.60 (included with system):** Required for setting SQA-V system defaults, archive management/data transfer, capture and storage of video images from the SQA-V and for displaying and printing self test data.
- **Excel/Word (required for V-Sperm GOLD)**

SECTION 2: System Overview

The SQA-V is a high performance analytical medical device that combines technology in electro-optics, computer algorithms and video microscopy. The system performs a 75-second semen analysis and has the ability to print test results and archive up to 500 patient records. The system is self-testing and self-calibrating and runs latex beads or stabilized sperm quality controls. Two systems: **Automated** and **visualization** allow the user the flexibility to analyze all types of semen samples.



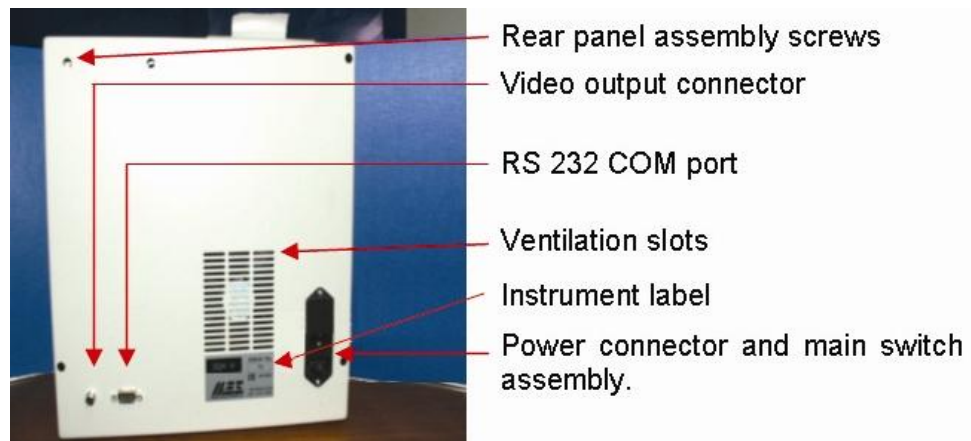
NOTE: The **TEST** button of the SQA-V keypad is only active in the **CALIBRATION** mode.

The **ARCHIVE** button on the keypad is inactive because the SQA-V archive is managed through V-Sperm GOLD.

Keypad Navigation

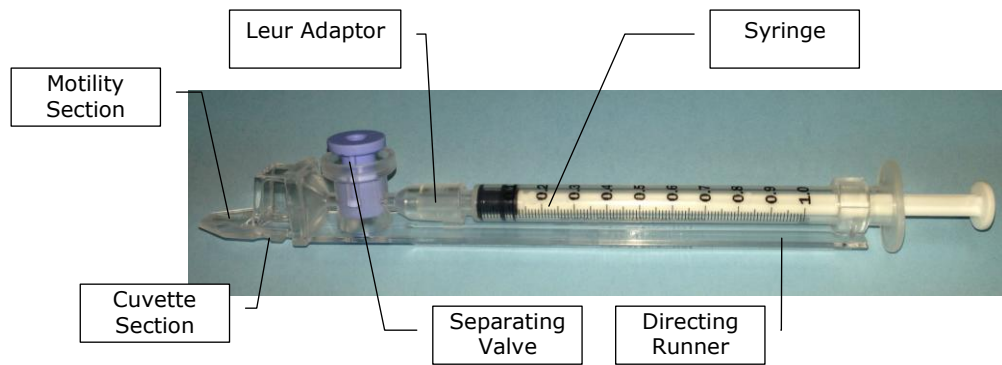
- Use **NUMERIC** keys to enter data; **ARROW** keys to move to the next field.
- Press **ENTER** to select menu options, confirm data entries and to move to the next screen or field.
- Use the **ESC** button to return to the previous screen or field.

Rear Panel



**SQA-V
Components**

Measurement Capillary



- Disposable, designed to collect and test samples in a biologically safe manner.
- Motility is measured in the 0.3 mm (thin) "Capillary Section." This section requires 20 micro liters of semen.
- Concentration is measured in the 10 mm (tall) "Cuvette Section." This section requires 450 microliters of semen.
- Both the measurement and visualization chambers of the SQA-V will accommodate the testing capillary. Refer to: "Filling the SQA-V Capillary with Normal and Low Volume Samples" in the Appendix section of this guide for instructions on how to use the SQA-V testing capillary.

Slide Adaptor

NOTE:

In order to accurately visualize the sample it must be centered approximately 12mm from the end of the glass slide.



- Use with a standard laboratory slide 76 x 25.6 mm and 22 x 22 mm cover-slip with a 10 µl sample placed approximately 12 mm from the end of the slide for accurate results.
- For use in the **visualization compartment** of the SQA-V.

Automated Test Results

Semen Parameters Reported by the SQA-V

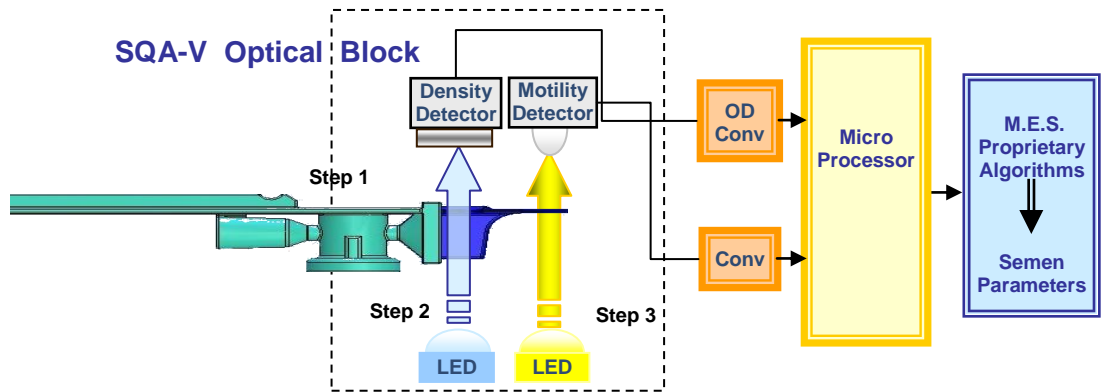
Semen Parameters with SQA-V Abbreviation in Brackets			
Sperm Concentration (SPERM CONC.)	M/ml	Velocity (VELOCITY)	mic /sec
Total Motility (TOTAL MOTILITY <PR+NP>)	%	Sperm Motility Index (SMI)	#
Progressive Motility (PROG. MOTILITY <PR>)	%	Total Sperm Number / ejaculate (SPERM #)	M
Non-progressive Motility (NONPROG. MOTILITY <NP>)	%	Total Motile Sperm / ejaculate (MOT. SPERM)	M
Immotility (IMMOTILITY <IM>)	%	Total Progressively Motile Sperm / ejaculate (PROG. SPERM)	M
Sperm Morphology (normal forms, %) (MORPH. NORM. FORMS, WHO 5 th)	%	Total Functional Sperm / ejaculate (FUNC. SPERM)	M
Motile Sperm Concentration (MSC)	M/ml	Total Morphologically Normal Sperm / ejaculate (MORPH. NORM. SPERM)	M
Progressively Motile Sperm Concentration (PMSC)	M/ml	Postvasectomy: Motile, Immotile and Total Sperm/Scan (#SPERM/SCAN: MOTILE, IMMOTILE and TOTAL)	#
Functional Sperm Concentration: Progressively Motile Sperm with Normal Morphology (FSC)	M/ml	Postvasectomy: Motile, Immotile and Total Sperm/sample volume (#SPERM/SAMPLE VOLUME: MOTILE, IMMOTILE AND TOTAL)	M

Table of the Reportable Range of the SQA-V

Reportable Range

REPORTABLE RANGE OF THE SQA-V Gold			
SAMPLE	SPERM CONC in M/ml	MSC in M/ml	Motility %
FRESH	2-400 or < 2 M/ml	0.2-400 or <0.2 M/ml	0-100%
WASHED	2-200 or < 2 M/ml	0.2-200 or <0.2 M/ml	0-100%
FROZEN	Not reported	0.2-200 or <0.2 M/ml	Not reported
POSTVASECTOMY	Manual Input	0-30 Sperm/Scan	Not reported

Technology SECTION 3: Technology



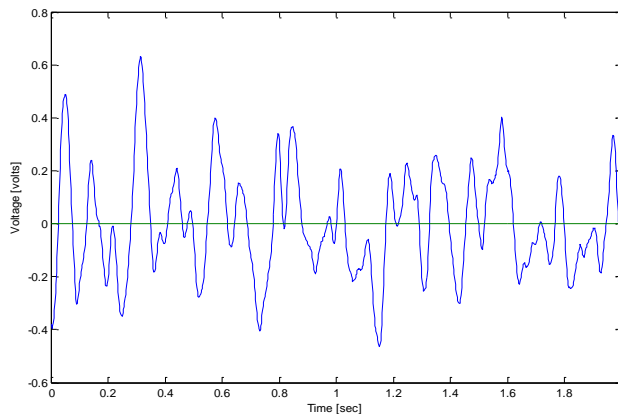
Step 1: The capillary is inserted into the measurement compartment.

Step 2: Concentration:

- Millions of sperm cells are analyzed: A very specific wavelength of light is absorbed by the sperm cells in the concentration chamber of the SQA-V testing capillary.
- An optical density detector measures the amount of light absorbed by the cells and converts it to optical density (OD).
- The "OD" reading is translated into sperm concentration by a microprocessor based on proprietary MES algorithms.

Step 3: Motility:

- Tens of thousands of sperm cells are analyzed in the thin section of the SQA-V capillary as they move through a light beam in the SQA-V: The movement of motile sperm cells causes light disturbances.
- These light disturbances are converted into electronic signals with "peaks and valleys."
- The electronic signal peaks are analyzed by microprocessor software based on a proprietary MES algorithm and translated into motility parameters.

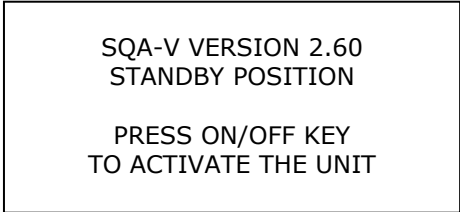


Electronic Signal of Motile Sperm

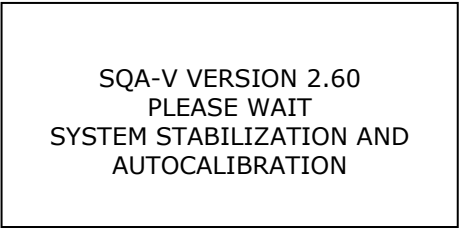
SECTION 4: Getting Started / Set-Up

Power-On

- Attach factory supplied electrical cable to the outlet on the rear panel.
- Plug cable into a grounded electrical source.
- Turn on SQA-V by pressing the main switch located on the rear panel. The **Power** indicator will illuminate and the following screen will be displayed.



Auto-Calibration and Self-Test

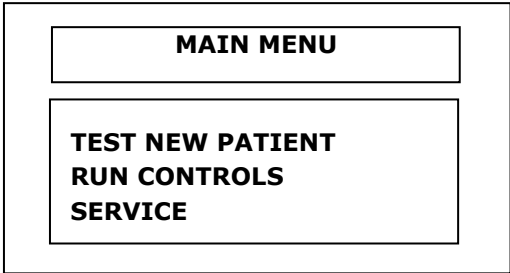


NOTE:

Do not insert a capillary/slide into the device during the stabilization process.

Do not use any of the keyboard functions during stabilization.

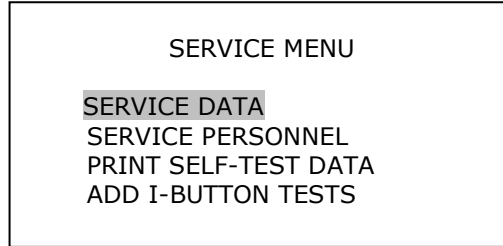
- Press **ON/OFF** key on the keypad and system stabilization and auto-calibration will begin.
- This process takes 5-7 minutes.
- When the system stabilization and auto-calibration processes are complete, a series of tests will be run.
- Do not insert a capillary/slide into the device or use any of the keyboard functions until instructed to do so by the system.
- The MAIN menu will appear when the self-test process is complete. The SQA-V is now ready for use.



Set-up System Defaults

SQA-V system defaults are set-up through V-Sperm GOLD software. Therefore a connection needs to be established between the SQA-V and the PC.

- From the **MAIN MENU**, select **SERVICE > SERVICE DATA**.



SERVICE DATA

1.	18	8.	112	15.	1.3
2.	5	9.	10	16.	110
3.	150	10.	6	17.	2
4.	28	11.	89	18.	1000
5.	77.65	12.	31	19.	1
6.	512	13.	100		
7.	0.000	14.	100		

- The RS232 communication cable must be connected to the SQA-V and the PC.
- Turn-on the PC and activate the V-Sperm GOLD version 3.60 software.
- From the V-Sperm GOLD main navigation screen select **SET-UP > SQA-V > SQA-V Defaults**. Then press the **CONTINUE** button.
- V-Sperm GOLD will display the SQA-V system set-up screen:

SQA-V set-up screen from V-Sperm GOLD

CONTROL set-up screen from V-Sperm GOLD

System

Choose date format <input checked="" type="radio"/> Europe (DD/MM/YY) <input type="radio"/> USA (MM/DD/YY)	SQA-V Date Enter local date <input type="text" value="9 / 3 / 2015"/>
Conc./Chamber Standard <input type="radio"/> 1 <input checked="" type="radio"/> 2	Printing Options <input checked="" type="checkbox"/> Automatically print all test results <input checked="" type="checkbox"/> Automatically print Self-Test Report on Start Up Printer Type: <input type="radio"/> Thermal <input checked="" type="radio"/> Ink
LES <input type="radio"/> 1 (US) <input checked="" type="radio"/> 2 (ROW)	

Control

Control Media
 Latex Beads Stabilized Sperm

Level 1			Level 2			Negative Control		
Lot #	1		Lot #	2		Lot #	3	
Exp. Date	08/15		Exp. Date	08/15		Exp. Date	08/15	
SQA-V	Target Value	+/- Range	SQA-V	Target Value	+/- Range	SQA-V	Target Value	+/- Range
	30	5		20	3		0.0	0.0

NOTE: All Set-up fields must have data in order to transfer information to the SQA-V. If CONTROL settings are not known, enter "0" LOT #/ Target Value +/- Range. Enter current date for the date field.

NOTE: The **Set-up** data transfer may take several minutes! Please wait.....

NOTE: Factory default settings are listed in **RED**.

Testing Samples

Patient Information

PLEASE NOTE:

The SQA-V is calibrated to run semen specimens at room temperature. It is not necessary nor will the user get accurate motility results if the sample is heated to 37°C.

- **SQA-V System Default settings:**
 - Date Format (DD/MM/YY) or (MM/DD/YY)
 - Local date setting
 - Conc./Chamber Standard 1 or 2 (See appendix section for more information).
 - LES setting: Check with your distributor for set-up
 - Printing options: automatically print test results/self test report on start-up.
- **Control Set-up (from the manufacturer's labeling):**
 - Select type of control: Latex beads or Stabilized Sperm CAP.
 - Enter Lot Number for each control level (enter "0" if not known).
 - Enter +/- Range for each control level (enter "0" if not known).
 - Enter EXPIRATION date (use current date if the EXP date is unknown).
- Press the **Report** button to view and print the selected default settings.
- Press **Apply** to accept the default settings and transfer them to the SQA-V.

SECTION 5: Testing Semen Samples

Information about the patient and sample is entered prior to the testing process. In order to accurately "classify" the semen sample by type and volume and understand the options for testing, refer to the information below.

Entering Patient and Sample Information

ENTER PATIENT / SAMPLE DATA	
PATIENT ID:	5788114
BIRTH DATE:	01/01/85
ABSTINENCE:	4 DAYS
SAMPLE PROCESSING	
SAMPLE / ACCESSION # 88	
COLLECTED:	DD/MM/YY HH:MM
RECEIVED:	DD/MM/YY HH:MM

- From the **MAIN MENU** select **TEST NEW PATIENT** and the **ENTER PATIENT/ SAMPLE DATA** screen is displayed.
- Enter the requested sample/patient information using the SQA-V keypad:
 - **PATIENT ID** – Unique number identifying the patient (Maximum of 20 numbers can be entered).
 - **BIRTH DATE** – Birth date of the patient.
 - **ABSTINENCE** – Number of days since the patient's last ejaculation.
 - **SAMPLE/ACCESSION #** – Up to 20 numbers identifying the sample.
 - **COLLECTED** – Date and time the sample was collected.
 - **RECEIVED** – Date and time the sample was received.

Sample Information

Press **ENTER** to view the next screen:

SAMPLE TYPE	
SELECT	FRESH / WASHED / FROZEN / POSTVASECTOMY
VOLUME	2.5 ml
WBC CONC.	SELECT < 1 M/ml OR >= 1 M/ml
PH	7.0
APPEARANCE	NORM./ABNORM.
VISCOSITY	NORM/ABNORM
LIQUEFACTION	NORM./ABNORM.

PLEASE NOTE:

1:2 dilution means 1 part semen volume plus 1 part diluent volume, which results in a 1:2 dilution. MES has included (1+1) to further define this dilution in order to prevent confusion.

PLEASE NOTE:

Refer to the appendix section of this user guide for information on how to measure semen WBC's and pH and how to handle viscous samples.

Sample Data

- Select: **SAMPLE TYPE** (required entry) based on the following options:
 - **FRESH** – Sample not enriched, diluted or treated and is within 1 hour of collection. Exception: Low volume samples diluted 1:2 (1+1) with QwickCheck dilution media can be used according to User Guide instructions.
 - **WASHED** – Sample enriched or prepared for artificial insemination using a commercial media to replace seminal plasma. Frozen samples containing egg yolk buffer are excluded.
 - **FROZEN** – Samples that have been frozen. Only motility parameters will be reported (MSC, PMSC, SMI and VELOCITY) in order to quantify the impact of freezing and thawing on the motility parameters of the specimen.
 - **POSTVASECTOMY** – Fresh samples designated as postvasectomy and tested within an hour of collection.
- Enter the remaining sample information using the SQA-V keypad:
 - **VOLUME** – Volume of the **whole** ejaculate in milliliters.
 - **WBC CONC.** – select < 1 M/ml (normal) OR >= 1 M/ml (abnormal) leukocytes (required entry). (QwickCheck Test Strips recommended).
 - **PH** – pH of the semen sample (QwickCheck Test Strips recommended).
 - **APPEARANCE** – NORM/ABNORM visual assessment of the specimen.
 - **VISCOSITY/LIQUEFACTION** – NORM/ABNORM (WHO 5th guidelines for NORM liquefaction is within 60 minutes of collection @ room temperature).
- If **POSTVASECTOMY SAMPLE TYPE** was selected, please refer to the section "Postvasectomy Test" in this user guide.

Sample Volume

IS SAMPLE VOLUME SUFFICIENT FOR COMPLETE TESTING >= .5 ml?

YES/NO

- After entering the patient and sample data, the screen above will be displayed.
- Using the left and right arrow keys and then **ENTER**, select:
 - **YES** for **NORMAL VOLUME** samples ≥0.5 ml.
 - **NO** for **LOW VOLUME** samples < 0.5 ml.

Low Volume Samples

Please note:

Prior to each running a test, the system will perform autocalibration (do not insert a capillary until instructed to do so on the screen.)

- If the sample is < 0.5 ml two options are available: Run as a low volume sample and obtain just motility parameters or dilute the sample 1:2 (1+1) with QwikCheck Dilution media and obtain a report of all parameters.
- To run a low volume sample: Aspirate only 20 µl of sample into the motility section of the capillary following the instructions in the Appendix section of this User Guide: "Filling the SQA-V Capillary with a Low Volume Samples".

LOW VOLUME SPECIMEN

PLEASE SELECT SAMPLE TESTING OPTION:
 DILUTE SEMEN 1:2 (1+1) WITH MEDIA
 LOW VOLUME – 20 MICROLITERS ONLY
 MOTILITY PARAMETERS ONLY

LOW VOLUME SAMPLE

FILL CAPILLARY – 20 MICROLITERS
 CLEAN AND WIPE CAPILLARY

INSERT CAPILLARY INTO CHAMBER

TEST RESULTS

MOTILITY PARAMETERS ONLY

MSC	18.5 M/ml	VELOCITY	5 mic/sec
PMSC	8.3 M/ml	SMI	26

TOTALS PER VOLUME

MOT SPERM	18.5M	PROG SPERM	8.3M
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Diluted Samples

Please note:

See the appendix section of this guide for information about dilution media.

- Or the low volume sample can be diluted 1:2(1+1) with QwikCheck-Dilution media:

LOW VOLUME SPECIMEN

PLEASE SELECT SAMPLE TESTING OPTION:
 DILUTE SEMEN 1:2 (1+1) WITH MEDIA
 LOW VOLUME – 20 MICROLITERS ONLY
 MOTILITY PARAMETERS ONLY

LOW VOLUME SPECIMEN

1. DILUTE SEMEN 1:2 (1+1) WITH MEDIA
2. MIX SAMPLE THOROUGHLY
3. FILL, CLEAN AND WIPE CAPILLARY

INSERT CAPILLARY INTO CHAMBER

- Follow the instructions in the appendix section of this User Guide: Filling the SQA-V Capillary with a Normal Volume Sample.
- The testing cycle and test results will be the same as a normal volume specimen (see screens below).

**Normal
Volume
Samples**

- The SQA-V algorithm compensates for the sample dilution as long as the sample has been diluted accurately (If the total sample volume is 0.4 ml then 0.4 ml of a clear media such as Earle’s buffer must be added).
- Recommendation: If the LOW VOLUME sample is viscous, first treat with the QwikCheck-Liquefaction kit and then dilute the sample for greater accuracy.

FRESH
NORMAL VOLUME SPECIMEN

1. MIX SAMPLE THOROUGHLY
2. FILL, CLEAN AND WIPE CAPILLARY
3. WAIT FOR AUTOCALIBRATION

AUTOCALIBRATION – DO NOT TOUCH UNIT

- If the sample was ≥ 0.5 ml the screen above will provide instructions for preparing a testing capillary.
- Fill the SQA-V testing capillary according to the instructions in the Appendix section of this user guide: “Filling the SQA-V Capillary with a Normal Volume Sample”.

FRESH
NORMAL VOLUME SPECIMEN

1. MIX SAMPLE THOROUGHLY
2. FILL, CLEAN AND WIPE CAPILLARY
3. WAIT FOR AUTOCALIBRATION

INSERT CAPILLARY INTO CHAMBER


- The screen above will be displayed when it is time to insert the filled testing capillary in the measurement compartment, testing will begin automatically.

PLEASE NOTE:


The SQA-V will begin testing when a capillary is placed into the testing chamber.

Testing

A sample is tested in approximately 75 seconds. If the sample is low quality, the system will perform an additional 2 minute test:

TESTING 

DO NOT MOVE CAPILLARY OR
OPERATE DEVICE DURING TESTING

TESTING 

LOW QUALITY SAMPLE

TESTING WILL TAKE 2 MORE MINUTES

Test Results

TEST RESULTS	
SPERM CONC.	32.6 M/ml
TOTAL MOTILITY <PR+NP>	28 %
PROG. MOTILITY <PR>	19 %
NONPROG. MOTILITY <NP>	9 %
IMMOTILITY <IM>	72 %
MORPH. NORM. FORMS, WHO 5 th	21 %

TEST RESULTS			
MSC	9.1 M/ml	FSC	2.5 M/ml
PMSC	6.3 M/ml	VELOCITY	9 mic/sec
SMI	34		
TOTALS PER VOLUME			
SPERM #	81.5 M	MOT. SPERM	22.8 M
PROG. SPERM	15.8 M	FUNC. SPERM	6.3 M
MORPH. NORM. SPERM	6.8 M		

Low Quality Test Results

- Low quality test results may be reported as < or > when one or more of the parameters falls below the SQA-V dynamic range. Only the following will be reported: Sperm Concentration, Motility, SMI and Motile Sperm Concentration due to the limited number of cells, very low motility and/or poor morphology.
- Examples of test results reported in this manner are seen in the screens below:

TEST RESULTS	
SPERM CONC.	2.7 M/ml
TOTAL MOTILITY <PR+NP>	< 5 %
PROG. MOTILITY <PR>	%
NONPROG. MOTILITY <NP>	%
IMMOTILITY <IM>	%
MORPH. NORM. FORMS, WHO 5 th	%

TEST RESULTS			
MSC	< 0.2 M/ml	FSC	M/ml
PMSC	M/ml	VELOCITY	mic/sec
SMI	0		
TOTALS PER VOLUME			
SPERM #	N.A.	MOT. SPERM	N.A.
PROG. SPERM	N.A.	FUNC SPERM	N.A.
MORPH. NORM. SPERM	N.A.		

- The test results will be saved/printed automatically or an option to save and print will be displayed depending on how the SQA-V was set-up.

**Printing
Saving and
Transferring
Test Results
to V-Sperm**

- If the SQA-V default was set to automatically print/save test results, the screen below will now be activated.

DATA SAVED AND
NOW PRINTING

- Immediately after saving/printing test results, an option to transfer the results of the test just completed to V-Sperm is displayed on the SQA-V.
- V-Sperm Gold must be activated and the PC must be connected via the RS232 cable to the SQA-V.
- Following the screen directions, simply press the "Import Test" main menu navigation button in V-Sperm and the test will automatically be transferred into the V-Sperm data base.

TO TRANSFER TEST RESULTS TO V-SPERM:

PRESS: "IMPORT TEST" BUTTON
IN V-SPERM

PLEASE NOTE:

The SQA-V archive is viewed from V-Sperm only. The archive must be transferred to the V-Sperm PC in order to view, delete and edit records.

- The archive of the SQA-V can accommodate 500 Patient Test records. A warning will appear when the archive is almost full. Data **MUST** be transferred to the PC or it will be lost, overwritten or the SQA-V will no longer permit testing.
Warning QC Tests are not saved in the SQA-V (there is no QC Archive). Transfer QC Tests to V-Sperm after performing each QC test by following the screen instructions (above screen) after the test is run.
- When the screen below is displayed, **Patient Records** must be transferred from the SQA-V archive to the PC:

ARCHIVE ALMOST FULL

TO AVOID POSSIBLE LOSS OF DATA
DOWNLOAD THE ARCHIVE TO THE PC

PRESS ENTER TO CONTINUE

- From the SQA-V, go to **MAIN MENU > SERVICE > SERVICE DATA**.
- Make sure the RS232 communication cable is connected between the SQA-V and the PC.
- Turn-on the PC and activate the V-Sperm GOLD version 3.60 software.
- From the V-Sperm main navigation screen select **IMPORT/EXPORT > IMPORT DATA > select IMPORT ARCHIVE (PATIENT RECORDS)**. Press **CONTINUE** and the records will automatically be transferred.
- After the records have been successfully transferred to V-Sperm, select **YES** on the next screen to delete the SQA-V (Patient) archive from the SQA-V.

Post Vasectomy Testing

Postvasectomy Test

The SQA-V runs a five minute POSTVASECTOMY test that can detect the presence of a very small number of motile cells. Once the automated test has been performed, the user is given the option to follow the POSTVASECTOMY protocol outlined below and "scan" the testing capillary in the SQA-V visualization system (A POSTVASECTOMY Protocol can also be found in the appendix section of this guide).

By scanning through the depth of the testing capillary, immotile and motile sperm cells can be readily identified, easily counted and entered in the operational screen for visual confirmation of the automated test results. Clinical studies positively demonstrated that by incorporating both the SQA-V automated AND visualization system in the testing protocol, a very high level of accuracy is obtained for identifying motile and non-motile sperm cells in POSTVASECTOMY samples.

In order to obtain similar levels of accuracy it is imperative that the user strictly follow the manufacturer's protocol outlined below. Additionally, once the testing cycle is completed, test results can be documented by capturing and archiving a video clip of the postvasectomy specimen using V-Sperm™ software.

Select **POSTVASECTOMY** as the SAMPLE TYPE from the ENTER PATIENT / SAMPLE DATA screen.

- Fill the SQA-V testing capillary following instructions in the appendix section of this guide: "Filling the SQA-V Capillary with a Normal Volume Sample."
- Insert the testing capillary into the SQA-V lower chamber when instructed. Testing will begin automatically.
- Testing takes approximately 5 minutes.
- Test results for motile sperm are reported.
- Select **YES** to when asked: "ENTER VISUAL DATA PER USER GUIDE?" to manually enter the number of MOTILE/IMMOTILE sperm seen on the visualization system.
- Press **ENTER** to continue.
- Take the same testing capillary and insert it into the visualization (upper) compartment.
- Set the magnification to x300 (Full zoom out) and press ENTER to continue.
- "Scan" the depth of the capillary by slightly turning the visualization focus knob (10 fields can be visualized) and enter the total # **MOTILE/IMMOTILE SPERM cells** visualized in all 10 fields.
- The SQA-V will automatically report the GREATER # of cells found by the Automated or Visualization system.
- Press **ENTER** and the test results screen will be displayed.

Please note:

The POSTVASECTOMY test takes approximately 5 minutes to run and is highly sensitive to motion. Please do not disturb the SQA-V or the testing capillary during the testing cycle or the results may be impacted.

TESTING

DO NOT MOVE CAPILLARY OR OPERATE DEVICE DURING TESTING

THIS TEST TAKES APPROX. 5 MINUTES

POSTVASECTOMY

SPERM/SCAN: # SPERM/SAMPLE VOL.:

MOTILE 3 MOTILE 0.2 M

ENTER VISUAL DATA PER USER GUIDE?

YES/NO

PLEASE INSERT CAPILLARY INTO VISUALIZATION SLOT
ADJUST MAGNIFICATION TO x300

PRESS ENTER

TURN FOCUS KNOB AND SCAN THROUGH ENTIRE CAPILLARY DEPTH TO COUNT MOTILE AND IMMOTILE SPERM

PLEASE ENTER:

MOTILE SPERM 3

IMMOTILE SPERM 8

- Leave the testing capillary in the visualization chamber and transfer the test results to V-Sperm to capture and attach a video clip of the sample in the patient's record.
- If the SQA-V reports > 30 motile spermatozoa, a screen will indicate that a NORMAL TEST should be run instead of a POSTVASECTOMY test.
- > 30 motile spermatozoa is equivalent to MSC > 2M/ml.

POSTVASECTOMY			
# SPERM/SCAN: MOTILE > 30			
PLEASE RE-RUN AS A NORMAL TEST			
POSTVASECTOMY			
# SPERM/SCAN:		# SPERM/SAMPLE VOL:	
MOTILE	3	MOTILE	0.2 M
IMMOTILE	8	IMMOTILE	0.5 M
TOTAL	11	TOTAL	0.7 M

Controls Set-Up and Testing

SECTION 6: Controls

Please note:

When a new control lot is used, the control default settings must be changed prior to initiating a test.

Refer to section(s):

Set-up: Assayed Control and

Set-up: Non Assayed material

External quality control samples (CONTROLS) are run on the RUN CONTROLS mode from the MAIN MENU of the SQA-V. Commercially available latex beads or stabilized sperm CAP can be run as non-assayed controls. QwikCheck™ beads produced by Medical Electronic Systems are assayed for the SQA-V. It is recommended that controls be run daily or based upon laboratory protocols.

The control media is aspirated into the testing capillary and run in the same manner as a normal volume specimen in the testing compartment of the SQA-V.

For each **new lot** of controls, SQA-V system defaults need to be set-up/updated through V-Sperm GOLD prior to running a test. To run an assayed control use the information for Target Value and +/- Range provided on the product labeling. To run a non-assayed control, the Target Value and +/- range must be established by the laboratory or set 0 (zero) if not established. Follow instructions below to **set-up** an assayed or non-assayed material. The testing process is the same.

Set-Up: Assayed Control

Each time a new lot of an assayed control is to be run, the user must set-up/update the CONTROL settings through V-Sperm GOLD as described below. Previous settings (defaults) will remain in place until updated.

- Step 1:** From the SQA-V **MAIN MENU** select **SERVICE > SERVICE DATA**.
- Step 2:** Make sure the SQA-V is connected to the PC via the RS232 communication cable.
- Step 3:** Activate the V-Sperm on the PC and select: **SET-UP > SQA-V** and press **CONTINUE**.
- Step 4:** The set-up screen below will be activated in V-Sperm GOLD on the PC:

Please note:

Level 1, 2, and NEGATIVE control set-up screen from V-Sperm GOLD.

The NEGATIVE control may also be labeled Level 3 control on the SQA-V.

For the SQA-V to work properly the CONTROLS must have set-up data inserted. If control material is not available or non-assayed enter current date in the EXP Date field and zeros in all other fields.

- Step 5:** Select the type of control (Latex Beads or Stabilized Sperm)
- Step 6:** Enter the following information from the box labeling:
 - **LOT#** - number identifying the control media lot.
 - **EXP. DATE** – control expiration date (MM = month, YY = year).
 - **TARGET VALUE and +/- Range** – manufacturer's "Target Value and +/- Range" for the SQA-V Automated System.
 - **NEGATIVE** control target values and +/- ranges are pre-set to 0.0.
- Step 7:** To save settings: Press **APPLY**. The set-up may take two minutes.

Please note:

To run 10 replicates: After each completed test, remove the capillary and initiate the CONTROL test again using the same capillary.

Running Controls in the SQA-V

Set-Up: Non-Assayed Material to Establish the target value and +/- range
This is also the set-up procedure for sperm concentration proficiency challenge

Follow the same **Steps 1-5** for "Set-up: Assayed Control" above.

- Step 6:** Enter the following information from the product labeling
 - **LOT#** - number identifying the control media lot.
 - **EXP. DATE** – control media expiration date (MM=month, YY=year).
- Step 7:** Enter the **TARGET VALUE and +/- Range for Level 1 and Level 2:**
 - Enter 00 for the target value.
 - Enter 0.0 for the +/- range.
 - NEGATIVE control target value and +/- range is pre-set to 0.0
- Step 8:** Save settings: Press **APPLY**. The set-up takes about two minutes.
- Step 9:** Establish the target value and +/- range for each level:
 - Fill a testing capillary and run 10 replicates following the instructions below "Control Testing."
 - Calculate the mean target value. Based on laboratory protocols determine the +/- range (Example: 2SD).
 - Follow steps 1-7 of "Set-Up: Assayed Control" to update the target value and +/- range for the control.

CONTROL Testing

- Select **RUN CONTROLS** from the **MAIN MENU** of the SQA-V.
- The Control defaults have already been set-up in V-Sperm.
- Select the **CONTROL LEVEL:** #1, #2 or NEGATIVE (LEVEL #3) that is being tested.
- Press **ENTER** to continue.
- Controls are run in exactly the same manner as a normal semen sample.
- Using control media, follow the same procedure for filling an SQA-V testing capillary with a NORMAL volume sample.
- Testing will begin automatically.
- Control test results will be displayed on the SQA-V screen.
- LOW, HIGH or NORM. will be displayed based on the testing outcomes vs. target value and +/- range (Disregard this for non-assayed controls target range set at "0").
- Test results will automatically be saved and printed.

<div style="border: 1px solid black; width: 100px; margin: 0 auto; padding: 2px;">MAIN MENU</div> <p>TEST NEW PATIENT RUN CONTROLS SERVICE</p>
<p>CONTROL LATEX BEADS SELECT: CONTROL LEVEL: LEVEL #1/LEVEL #2/NEGATIVE CONTROL</p> <p>PRESS ENTER TO CONTINUE</p>
<p>CONTROL: LATEX BEADS, LEVEL #1</p> <p>FILL, CLEAN AND WIPE CAPILLARY INSERT IN CHAMBER TESTING WILL BEGIN AUTOMATICALLY</p>
<p>CONTROL TEST RESULTS</p> <p>DATE 01/12/06 DD/MM/YY TIME 15:09:08 LEVEL #1 LOT# 11223344556677889900 EXP. DATE 04/09 MM/YY TYPE: LATEX BEADS TARGET VALUE: 45.0 +/- 6.3 M/ml CONC. RESULTS: 45.4 M/ml NORM. ACCEPTABLE RANGE: 38.7 – 51.3 M/ml</p>

Electronic Self-Test and Auto Calibration

The SQA-V automatically runs a series of tests to check calibration settings and the internal operating system. Tests are run when the system is turned on and prior to testing a sample.

Start-up:

- **Stabilization and auto calibration:** Checks system stability and reference ranges. The system sensors are analyzed for several minutes to insure that the values are within a very narrow acceptable range. Once the system is stable for 30 seconds it will pass stabilization and auto calibration. The system will fail if it is not stable for at least 30 seconds and a warning message will be displayed.
- **System noise:** Measures the electronic noise level of the system to insure effective measurement of electronic signals.
- **Self-test:** The system produces electronic signals that simulate motility and concentration measurements in order to check the performance of the system and verify that the calibration settings are consistent with the factory specifications. The SQA-V will report failures (see section on error and warning messages) and "freeze" the system if the system is not within the established self-test ranges.

Prior to testing a sample:

- **Auto calibration verification:** Reference values are read again. The electronic parameters of the concentration and motility channels are measured (without a testing capillary).
- **System noise:** Measures the electronic noise level of the system to insure effective measurement of electronic signals. Prior to running a test, the SQA-V will automatically adjust the noise level thresholds to insure accurate readings.
- **Electronic spikes:** Checks for any measurement points that are out of range electronically. More than three such points will fault the system and a warning message will be displayed.

Instructions for printing the SQA-V system parameters to prepare for technical support:

How to print a copy of the system parameters FROM THE SQA-V:

- Remove the testing capillary from the system.
- When a **FAILED SELF TEST** message appears select: **MAIN MENU > SERVICE>PRINT SELF-TEST DATA.**
- Press **ENTER** to generate a report.

How to view/print a copy of the system parameters FROM V-SPERM GOLD:

- Verify that the SQA-V is connected to the PC and V-Sperm is activated.
- From the SQA-V activate: **MAIN MENU > SERVICE > SERVICE DATA.**
- Select the V-Sperm navigation buttons: **UTILITIES>SELF-TEST DATA** and click **CONTINUE.**
- Click on the **PRINT** button to view a Service Data Report.
- Click **PRINT** in the upper left hand corner of the screen to print a report.

- Refer to the table below. Enter numbers in the "SQA-V Value" column that corresponds to the SQA-V system parameters printout. Compare the values. If the value from the SQA-V is within range mark the "Pass" column. If not, mark the "Fail" column.

#	Parameter	S/W Ver. 2.60	SQA-V Value	Pass	Fail
1.	Ref 1	150 – 400 mV			
2.	LED Cur 1	5 – 25 mA			
3.	Amplitude	50 – 100 mV			
4.	Zero Level	500 - 525			
5.	Ref 2	2500 – 3500 mV			
6.	LED Cur 2	10 – 32 mA			
7.	CONC. 1	0 – 1 M/ml			
8.	CONC. 2	50-150 M/ml			
9.	CONC. 3	300-600 M/ml			
10.	Count (Service Data, Item #12)	26 - 36			

Archive SECTION 7: Transferring the SQA-V Archive to V-Sperm

The SQA-V automatically prints PATIENT and CONTROL test results when a test is completed. Only PATIENT TEST results (not CONTROL test results) are saved in the SQA-V archive when the testing cycle is complete. **To view, navigate, edit and delete records, the test results have to be transferred to V-Sperm** immediately after running a test (on-line transfer) or imported to V-Sperm as a group (Patient tests only). The SQA-V can store 500 patient records but does not store the control records.

The screen below will be displayed when the PATIENT archive of the SQA-V is almost full:

ARCHIVE ALMOST FULL
TO AVOID POSSIBLE LOSS OF DATA
DOWNLOAD ARCHIVE TO PC
PRESS ENTER TO CONTINUE

The following screen will appear after every CONTROL TEST is performed:

ATTENTION!
TO AVOID LOSS OF DATA
TRANSFER CONTROL RESULTS TO V-SPERM
PRESS: "IMPORT TEST" BUTTON
IN V-SPERM

To transfer data to V-Sperm, first connect the SQA-V to the PC and activate the V-Sperm software. There are two options for transferring test results to V-Sperm:

IMPORT TEST RESULTS ON-LINE:

- Immediately after saving/printing test results, an option to transfer the results of the test just completed is displayed on the SQA-V.
- Following the screen directions, simply select the "Import Test" main menu navigation button in V-Sperm and the test will automatically be transferred into the V-Sperm data base.

TO TRANSFER TEST RESULTS TO V-SPERM:
PRESS: "IMPORT TEST" BUTTON
IN V-SPERM

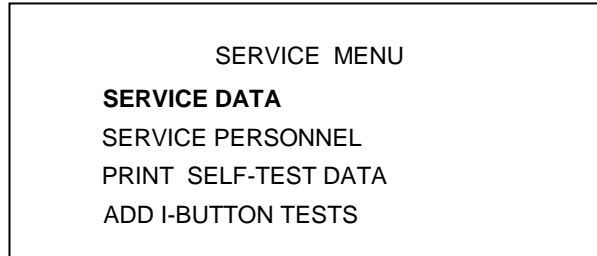
IMPORT PATIENT RECORDS FROM THE SQA-V TO V-SPERM:

- Select the V-Sperm navigation button: **IMPORT/EXPORT**.
- Select: **IMPORT DATA > IMPORT ARCHIVE** and press **CONTINUE** and the tests will automatically be transferred.
- Select: **YES** on the next screen to delete records from the SQA-V archive.

**Service
Menus**

SECTION 8: Service Menu

System set-up, maintenance and calibration can be performed from the SERVICE MENU. To activate this screen, press **SERVICE** in the MAIN MENU.



Service Data

Communication between the SQA-V and a PC via the RS232 interface is established through the SERVICE DATA screen. System set-up and upgrades are also performed through this screen.

The SQA-V archive can be transferred to a PC only when this screen is activated.

Service Personnel

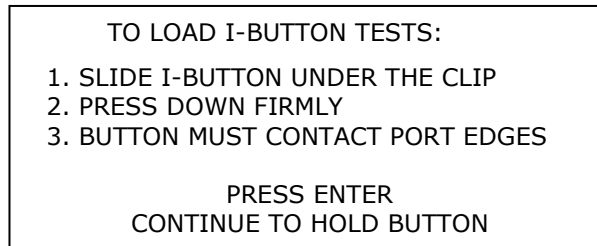
A **code** is required to access SERVICE PERSONNEL. This option allows a qualified service technician to access calibration and maintenance settings.

Print SQA-V SELF-TEST DATA

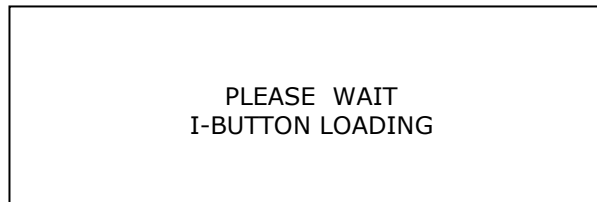
The system SELF-TEST DATA can be printed from this option.

Add I-Button Tests

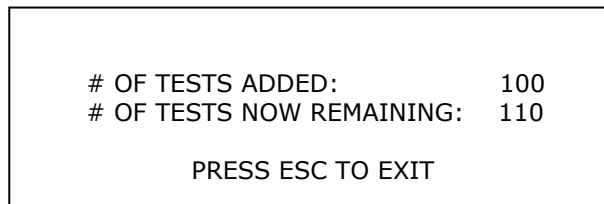
From the SQA-V **MAIN MENU** SELECT: **SERVICE > ADD I-BUTTON TESTS** and follow the on screen instructions seen below:



The I-Button loading screen below will appear:



The screen below will be displayed after successfully loading tests:



Please note:
Go to the SQA-V to load I-button tests directly to the device.

Visualization System

Operating Instructions

SECTION 9: Operating the Visualization System (Video Display)

The SQA-V Visualization System with video display (upper screen) is used to view and count sperm cells. The visualization system is a critical "link" to V-Sperm GOLD where enhanced, real time video can be displayed on a PC monitor. The visualization system:

- Accommodates both an SQA-V testing capillary to "scan" through a depth of 300 microns or a standard slide to view samples (20 micron depth).
- Operates via control knobs to set focus, brightness, contrast and color, and via the keypad zoom, illumination, and monitor on/off functions.
- Magnification range: x300 to x500.

Standard Slide Preparation:

- Use 10 µl of semen.
- Standard slide, 22 mm x 22 mm cover-slip (to insure 20 micron depth).
- Load the prepared, standard slide into the SQA-V slide adaptor.

Testing Capillary Preparation:

- Fill the SQA-V testing capillary for either a normal or low volume specimen (see Appendix).

Visualization Process:

- The video display will automatically illuminate when the SQA-V is turned on.
- Use monitor **ON/OFF** key on the keypad to independently operate the video display.
- Wait for the self-test to complete (system is disabled at this time).
- To ensure that the visualization system is working properly prior to use:
 - Press the **HIGH ILLUMINATION** key multiple times to ensure a maximum level setting.
 - **To view cells:** Press **ZOOM IN** to maximum magnification (x500).
 - **To count cells:** Press **ZOOM OUT** to minimum magnification (x300).
- Insert semen sample (either capillary or slide) into the visualization chamber.
- Adjust **CONTRAST, COLOR, BRIGHTNESS, FOCUS** and object **ILLUMINATION** controls for optimal image quality.
- Use **ZOOM OUT** (x300) / **ZOOM IN** (x500) to regulate magnification.

Counting Cells Using the Visualization Screen:

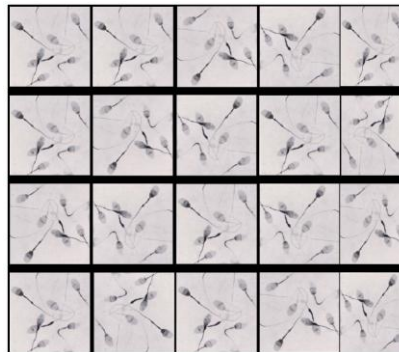
1. Follow the WHO Manual instructions for semen sample collection and preparation. Thoroughly mix the sample before step #2.
2. Pipette 10uL of the semen sample onto a standard slide and cover with a 22x22 mm coverslip. Prepare a new slide if air bubbles or liquid spillage occurs.
3. Load the slide into the slide adaptor and then insert the slide adaptor into the SQA-V visualization chamber. (Refer to the SQA-V User Guide APPENDIX 3: Using Standard Slides in the Visualization System for details).
4. Press the ZOOM-OUT button on the SQA-V keypad all the way to set the magnification to x300.

Please note:

The visualization screen grid of the SQA-V is calibrated to a CONC STANDARD default of "1" or Makler/non-dilutional chambers.

Please see the Appendix Section "Concentration Standard – Counting Chamber" for details.

5. Set the: BRIGHTNESS, CONTRAST & COLOR knobs of the video display:
 - a. COLOR knob: Turn clockwise to the end (maximum color),
 - b. CONTRAST: Turn counterclockwise to the end (maximum contrast),
 - c. BRIGHTNESS knob: Turn clockwise from the darkest setting until the background is light (not maximum!).
6. Adjust the focus knob to maximize the image: Turn clockwise all the way. Then turn counterclockwise until a clear image appears on the screen.
7. Go to V-Sperm and click on the **Real Time Video** button. FREEZE the image.
8. The screen of both the SQA-V and the V-Sperm is divided into a grid containing 20-distinct squares (see below).



9. Each spermatozoon seen on the ENTIRE 20-square grid is 1 Million/ml of sperm concentration. FOR EXAMPLE: In the grid above, there are 7 spermatozoa in each cell of the grid. 7 (spermatozoa) X 20 (cells) = 140 M/ml sperm concentration for this sample.
10. To count a minimum of 200 cells (per WHO), turn the silver knob of the slide adaptor and a new field of view will be displayed in the grid.
11. When viewing multiple fields, divide the final count by the number of screens (fields of view) counted. For example, if two of the screens above are counted there would be a total number of 280 sperm cells so the sperm concentration will be: $280 \div 2 = 140 \text{ M/ml}$.
12. Refer to table 2.2 of the WHO Manual 5th Edition to determine if the duplicate counts are acceptable.

**Error and
Warning
Messages**

SECTION 10: Error Messages, Warning Messages and General Warning

General Warning:

- The SQA-V equipment’s built-in protection for the operator and the environment is ONLY operational if the SQA-V is operated properly following the manufacturer’s specifications.
- **CAUTION:** There is a risk of explosion or shorting if the SQA-V battery is replaced by an incorrect type. Replacement batteries MUST be the same type and manufacturer. Dispose of used batteries in accordance with the manufacturer instructions.
- Environmental condition for storage and transport: Recommended to store the SQA-V at temperatures between 20°C -30°C.
- Following the manufacturer’s recommended use, the expected life span of the SQA-V is a minimum of 5 years. The life span can be extended when utilizing the manufacturer’s annual preventative maintenance plan.

Stabilization Failed:

```
STABILIZATION FAILED
TURN OFF MAIN SWITCH ON REAR PANEL
REACTIVATE UNIT
IF PROBLEM PERSISTS,
CALL FOR TECHNICAL SUPPORT
```

- Ensure there is no testing capillary in the measurement compartment.
- Remove the SQA-V from sources of electronic noise and vibrations.
- Clean measurement compartment (refer to Appendix).
- Reboot the SQA-V without a testing capillary in the chamber:
 - Turn system **OFF** then back **ON** at the main switch on the rear panel.
 - Press the front panel **ON/OFF** key to begin Auto-Calibration/Stabilization.
- Call technical support if failure recurs.

Self-test Failed:

```
FAILED SELF-TEST
TURN OFF MAIN SWITCH ON REAR PANEL.
CLEAN OPTICAL CHAMBER.
REACTIVATE UNIT.
IF PROBLEM PERSISTS
CALL FOR TECHNICAL SUPPORT
```

- Ensure there is no testing capillary in the measurement compartment.
- Remove the SQA-V from sources of electronic noise and vibrations.
- Clean measurement compartment (refer to Appendix).
- Reboot the SQA-V without a testing capillary in the chamber:

- Turn the system **OFF** then back **ON** at the main switch on the rear panel.
- Press the front panel **ON/OFF** key to begin Auto-Calibration/Stabilization.
- Call technical support if this message is displayed again. Prepare for technical support by printing a copy of the SQA-V **SERVICE DATA**:
 - Press the SERVICE key on the SQA-V keypad to activate the **SERVICE MENU** screen.
 - Select: **PRINT SELF TEST DATA.**
 - Press **ENTER.**

Electronic Noise:

ELECTRONIC NOISE.
TURN OFF MAIN SWITCH ON REAR PANEL.
REACTIVATE UNIT.
IF PROBLEM PERSISTS,
CALL FOR TECHNICAL SUPPORT

- Ensure there is no testing capillary in the measurement compartment.
- Remove SQA-V from sources of electronic noise and vibrations (centrifuge).
- Clean measurement compartment (refer to Appendix) and after cleaning:
 - Turn the system **OFF** then back **ON** at the main switch on the rear panel.
 - Press the front panel **ON/OFF** key to begin Auto-Calibration/Stabilization.
- From the main menu: Select **TEST NEW PATIENT** and rerun the test.
- Call technical support if this message is displayed again. Prepare for technical support by printing a copy of the SQA-V **SERVICE DATA**:
 - Press the SERVICE key on the SQA-V keypad to activate the **SERVICE MENU** screen.
 - Select: **PRINT SELF TEST DATA.**
 - Press: **ENTER.**

Concentration Out of Range

Testing Semen Sample:

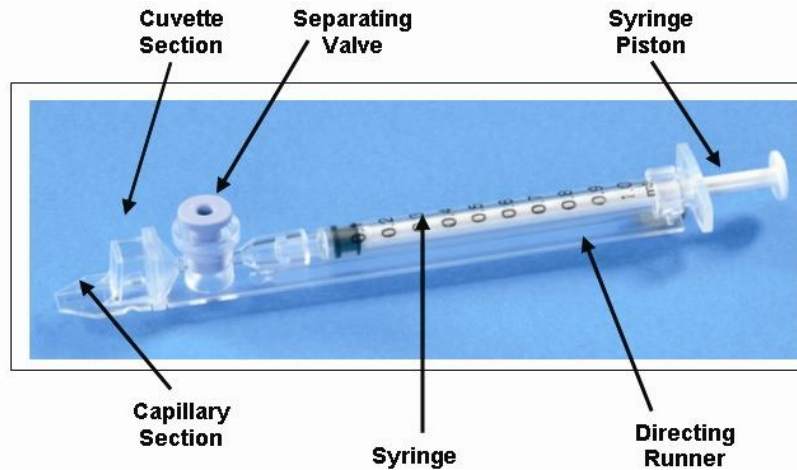
TEST RESULTS
OUT OF PHYSIOLOGICAL
RANGE
RETEST SAMPLE?
YES/NO

- A message will appear indicating that the tests results for Sperm Conc and/or MSC are beyond the upper limits of the dynamic range established by the manufacturer for testing. This message will appear if the SQA-V reads:
 - SPERM CONC > 500 M/ml or MSC > 450 M/ml
- Review sample handling technique (Appendix "Filling the SQA-V Capillary").
- Re-test the sample in a new SQA-V capillary. If the message appears again,

reboot the system.

- Call for technical assistance if problem persists.

APPENDIX 1: Filling the SQA-V Capillary with a Normal Volume Sample



Sample size, collection container and preparation:

1. Sample volume should be **at least .5 ml**. If sample volume is less than .5 ml see Appendix 2.
2. Sample container should be **wide-necked and deep enough** to facilitate inserting the capillary into the sample at the bottom of the container.
3. The semen sample must be **completely liquefied and well mixed prior to aspiration**. Gently rotate container to fully mix liquefied specimen.

WARNING: Do not shake nor use a pipette to aspirate and dispense specimen in order to mix, otherwise air bubbles will form.



Figure 1

4. **Carefully check that liquefied, fully mixed specimen is free of air bubbles** (or that there is an adequate amount of sample below the air bubbles) before immersing the capillary into the specimen, thus ensuring that no air bubbles will be aspirated into the capillary.

Filling the capillary:

1. **Push the syringe piston in fully.** Place only thin part of the capillary into the bottom of the sample while angling the sample container at about 45 degrees (Figure 1).
2. Placing two fingers below the piston head **pull the piston back slowly while keeping the tip of the capillary well below the sample level and below any surface bubbles** (Figure 1). Continue to aspirate the sample until it appears in the Luer adaptor.



Figure 2

NOTE: Transferring the sample to a standard "tissue culture dish" (3 cm in diameter/1 cm deep) will allow better visual control when filling the capillary as an intermediate step (see Figure 2).

3. Holding the capillary in a vertical position (Figure 3), **visually confirm that the sample has completely filled** the thin section (without a meniscus) and the cuvette section and appears in the Luer adaptor. **Tap on the syringe to make sure there are no air bubbles** in the sample. If, after tapping, some air bubbles appear below the Luer adaptor, dip the capillary into the semen sample again and aspirate a small quantity of semen to draw the air bubbles into the syringe.
4. Quickly (to avoid wicking) and **thoroughly wipe the outer surface of the capillary** - both top and bottom (Figure 4) with a delicate wipe (Kimwipes, etc.). It is important to remove all semen from the exterior of the capillary in order to prevent the SQA-V optical chamber from becoming clogged. Visually confirm that the capillary chambers are still full following the cleaning process. If some of the sample has been depleted (meniscus formed in the thin part of the capillary) fill the capillary part from the cuvette section by slightly pushing in the piston.



Figure 4



Figure 3

5. Slowly and carefully **push-in the separating valve** until it is level with the plastic (Figure 5). The capillary is now ready to be inserted into one of the SQA-V compartments for testing or viewing.



Figure 5

6. **For automated testing push the testing capillary into the lower measurement compartment with the blue stopper down.** Push it in as far as it will go to ensure that the capillary is properly seated in the compartment.
7. **To visualize the specimen, insert the capillary into the visualization compartment with the blue stopper up.**



APPENDIX 2: Filling the SQA-V Capillary with a Low Volume Sample

Sample size, collection container and preparation:

1. A sample as small as 20 micro liters can be tested for motility parameters by filling **ONLY** the thin section of the testing capillary (Figure 1).
2. The semen sample must be **completely liquefied and well mixed prior to aspiration**. Gently rotate the container to fully mix the liquefied specimen. **WARNING:** Do not shake nor use a pipette to aspirate and dispense specimen in order to mix, otherwise air bubbles will form.
3. **Carefully check that the liquefied, fully mixed specimen is free of air bubbles** (or that there is an adequate amount of sample below the air bubbles) before immersing the capillary into the specimen, thus ensuring that no air bubbles will be aspirated into the capillary.
4. **It is recommended that the sample be withdrawn from a standard "tissue culture dish"** (3 cm in diameter/1 cm deep) to allow for better visual control when filling the capillary.



Figure 2



Figure 1

Filling the capillary:

1. **Push the syringe piston in fully**. Place only the thin part of the capillary into the bottom of the sample (Figure 1).
2. **Pull the piston back slowly** without withdrawing the capillary from the sample. **Fill only the (thin) capillary chamber** with 20 micro liters of semen (Figure 1). The exact quantity aspirated can be determined by the gradations on the 1 ml syringe. Aspirate the sample until it just appears in the cuvette part while keeping the tip of the capillary well below the sample level and well below the level of any bubbles covering the liquid. Withdraw the capillary tip from the semen sample and visually inspect the capillary to ensure that the sample has completely filled the thin section (no meniscus).
3. Quickly (to avoid wicking) and **thoroughly wipe the outer surface of the capillary** - both top and bottom with a delicate wipe (Kimwipes, etc.). It is important to remove all semen from the exterior of the capillary in order to prevent the SQA-V optical chamber from becoming clogged. Visually confirm that the thin chamber of the capillary is still full of semen after completing the cleaning process. If some of the sample has been depleted push-in the piston slightly until the first drop appears on the capillary tip and then fill the capillary again from the sample container.
4. The separating valve must now be removed. Detach the entire syringe from the hub (Figure 2) and use the syringe tip to firmly **push-out the separating valve** from the underside of the capillary (Figure 3). Completely detach the separating valve (Figure 4). The capillary is now ready to be inserted into the SQA-V.
5. **PLEASE NOTE: Test Low Volume samples as soon as the sample is aspirated into the capillary.**



Figure 3



Figure 4

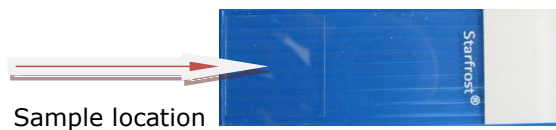
APPENDIX 3: Using Standard Slides in the Visualization System

Introduction

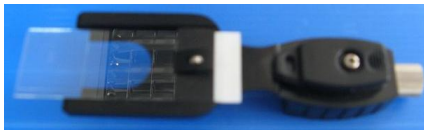
The SQA-V has a specially designed slide adaptor that enables the user to use standard slides to view semen samples in the SQA-V visualization compartment. A slide is "seated" in a stable and secure manner as described below and the slide adaptor is inserted into the SQA-V for testing.

User Instructions:

1. The slide adapter is designed for standard laboratory slides that are 76 mm long and 25.6 mm wide. Thickness may vary from 1 mm to 2 mm. The viewing section of the slide must be completely transparent.
2. Center a 10 micro-liter drop of semen at a distance of approximately 12 mm from the edge of the slide and cover with a standard (22 mm x 22 mm) cover-slip. The droplet of semen should be evenly spread across the entire surface area of the cover-slip automatically, without any additional pressure applied to the cover-slip:



3. Carefully place the prepared slide into the slide adapter (with the non-loaded side towards the slide holder):



4. Open the spring loaded slide holder by pressing on its outer edge. Slip the slide into the holder and release the spring:



5. Align the edge of the slide with the distal edge of the slide adapter by turning the silver slide position adjuster as seen below. The slide will now be firmly in place in the slide adapter:



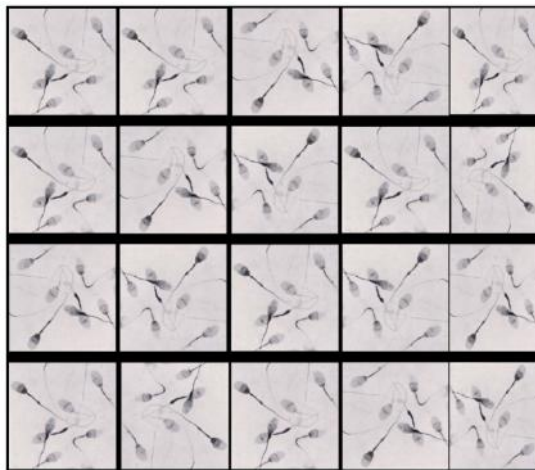
6. Insert the fully loaded slide adapter into the visualization chamber of the SQA-V:



7. Optimize the video image in the usual manner (please see the SECTION 9: Operating the Visualization System) and move to additional fields of view by turning the silver knob of the slide adapter.

APPENDIX 4: Counting Cells using the SQA-V Visualization System

1. Follow the WHO Manual instructions for semen sample collection and preparation. Thoroughly mix the sample before step #2.
2. Pipette 10uL of the semen sample onto a standard slide and cover with a 22x22 mm cover slip. Prepare a new slide if air bubbles or liquid spillage occurs.
3. Load the slide into the slide adaptor and then insert the slide adaptor into the SQA-V visualization chamber. (Refer to the SQA-V User Guide APPENDIX 3: Using Standard Slides in the Visualization System for details).
4. Press the ZOOM-OUT button on the SQA-V keypad all the way to set the magnification to x300.
5. Set the: BRIGHTNESS, CONTRAST & COLOR knobs of the video display:
 - a. COLOR knob: Turn clockwise to the end (maximum color),
 - b. CONTRAST: Turn counterclockwise to the end (maximum contrast),
 - c. BRIGHTNESS knob: Turn clockwise from the darkest setting until the background is light (not maximum!).
6. Adjust the focus knob to maximize the image: Turn clockwise all the way. Then turn counterclockwise until a clear image appears on the screen.
7. Go to V-Sperm and click on the **Real Time Video** button. FREEZE the image.
8. The screen of both the SQA-V and the V-Sperm is divided into a grid containing 20-distinct squares (see below).



9. Each spermatozoon seen on the ENTIRE 20-square grid is 1 Million/ml of sperm concentration. FOR EXAMPLE: In the grid above, there are 7 spermatozoa in each cell of the grid. 7 (spermatozoa) X 20 (cells) = 140 M/ml sperm concentration for this sample.
10. To count a minimum of 200 cells (per WHO), turn the silver knob of the slide adaptor and a new field of view will be displayed in the grid.
11. When viewing multiple fields, divide the final count by the number of screens (fields of view) counted. For example, if two of the screens above are counted there would be a total number of 280 sperm cells so the sperm concentration will be: $280 \div 2 = 140$ M/ml.
12. Refer to table 2.2 of the WHO Manual 5th Edition to determine if the duplicate counts are acceptable.

APPENDIX 5: Cleaning the Capillary Compartment

When to clean: **DAILY (step 1), WEEKLY (step 2)**

- Or if SELF-TEST or any other failure occurs
- Or if System becomes contaminated with semen

Cleaning kit components:

- Long cleaning brush
- Fibrous material cleaning paddles (single use)
- Sponge-tipped drying paddles (single use)
- Cleaning fluid (single drop dispenser)

PLEASE NOTE: Cleaning and drying Paddles are for ONE TIME use only!

CLEANING: STEP 1 (DAILY)

- Insert the long brush (bristle side down) into the upper portion of the lower chamber of the SQA in the same manner as a testing capillary (Fig 1 and 2).
- Pull the brush out, applying downward pressure to sweep or 'dust off' the optics (you will feel a 'shelf' in the back/top section of the chamber) – (Fig 2 and 3)
- **Monitor the system's "REF. 2" parameter. It should be between 2800 and 3200 mV if possible.**

CLEANING: STEP 2 (WEEKLY)

1. Use a **Fibrous material** cleaning paddle (fig 4)
 - Moisten with only **ONE** drop of cleaning fluid.
 - Shake off excess fluid.
 - Insert into the measurement compartment fibrous material facing **DOWN ONLY** (fig 5)
 - Move the cleaning capillary in and out three times.
2. Use a sponge-tipped drying paddle into the testing chamber and leave it for 10 – 15 seconds (fig 6).

NOTE: Do not move this drying paddle in and out.

Fig. 1: Long cleaning brush



Fig. 2: Cleaning lower chamber



Fig. 3: "Dusting off"



Fig. 4: **FIBROUS** cleaning paddle



Fig. 5: Insertion-**Fibrous material facing DOWN**



Fig. 6: Insertion of drying Paddle

APPENDIX 6: Reference Values of Semen Variables

SEMEN PARAMETER	SQA-V TEST NAME	REFERENCE RANGE*	SOURCE
Sperm Concentration (Count)	SPERM CONC.	≥15 M/ml	WHO 5 th manual*
Total Motility (PR+NP)	TOTAL MOTILITY <PR+NP>	≥40 %	WHO 5 th manual*
Progressive Motility (PR)	PROG. MOTILITY <PR>	≥32 %	WHO 5 th manual*
Non-progressive Motility (NP)	NONPROG. MOTILITY <NP>	-	-
Immotility (IM)	IMMOTILITY <IM>	-	-
Sperm Morphology (normal forms, %)	MORPH. NORM FORMS, WHO 5 th	≥4%	WHO 5 th manual*
Motile Sperm Concentration	MSC	≥6 M/ml	MES*
Progressively Motile Sperm Concentration	PMSC	≥5 M/ml	MES*
Functional Sperm Concentration	FSC	-	-
Velocity (Average path velocity – VAP)	VELOCITY	≥5 mic./sec.	MES*
Sperm Motility Index	SMI	≥80	MES*
Total Sperm Number	SPERM #	≥39 M	WHO 5 th manual*
Total Motile Sperm	MOT. SPERM	≥16 M	MES*
Total Progressively Motile Sperm	PROG. SPERM	≥12 M	MES*
Total Functional Sperm	FUNC. SPERM	-	-
Total Morphologically Normal Sperm	MORPH. NORM. SPERM	≥2 M	MES*

The ranges established above are based on WHO 5th reference values or MES (for proprietary semen parameters).

APPENDIX 7: Measuring WBC's in Semen

SQA-V Visualization System

Follow directions for preparing a standard slide with 10 µl of semen and refer to the "Using the Visualization System" section of this guide. View up to 10 fields by turning the silver slide adaptor knob. Search for leukocytes. If >1 M/ml are seen on the visualization system, select ABNORMAL (ABNORM) in the SAMPLE DATA screen.

QwikCheck™ Test Strips for Semen

Place one drop of semen on the test patch for WBC's (leukocytes) and follow the instructions on the TEST STRIP label/insert. Compare the patch to the color scale for WBC on the container. If the patch exceeds the darkest lavender color on the scale it indicates that WBC concentration in the sample is abnormal or >1 Million/ml.

NOTE: Test strips are also supported for pH testing of semen.

Clinical Trial

The WBC patch of the test strip changes color due to a chemical reaction caused by the presence of esterase in granulocytes. Esterases cleave to indoxyl ester, liberating the indoxyl which then reacts to diasonium salt to produce a violet dye. This chemical reaction is not affected by bacteria, trichomonads or erythrocytes present in the specimen.

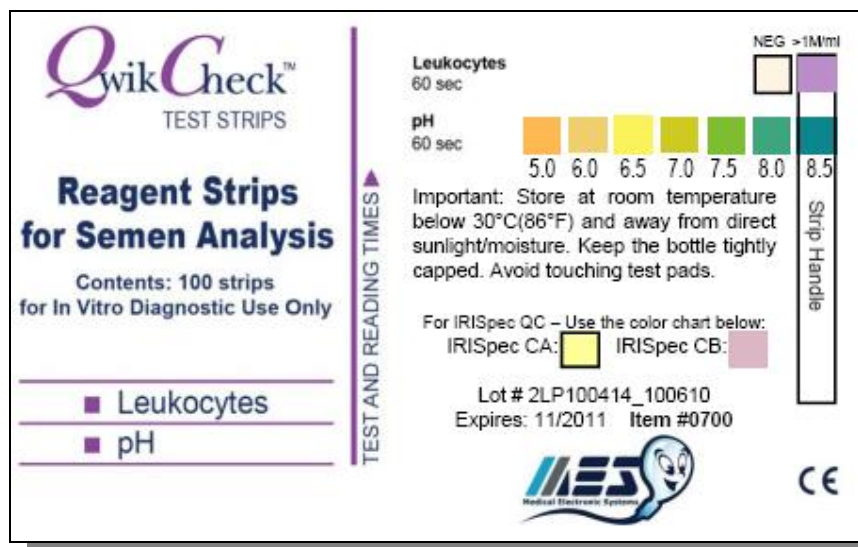
QwikCheck™ test strips were evaluated by Medical Electronic Systems Ltd. (MES) for use as a qualitative indicator (WBC's >1M/ml) of WBC's in human semen. To test this application WBC's were isolated from blood and re-suspended in seminal plasma. Varying concentrations of WBC's in seminal plasma were tested using the test strips. Test results were analyzed visually and by spectrophotometer readings.

Results and Conclusion

When the WBC concentration in semen is >1 Million/ml the WBC patch of the QwikCheck™ test strips exceeds the darkest lavender color on the color chart after the testing time. (This reading corresponds to WBC concentration > 1 Million/ml that is considered abnormal according to WHO 2010 5th edition, Pg. 107). A NEG includes both the NEG color on the label AND any color of lavender LIGHTER than the >1M patch on the label.

References

WHO 2010 5TH edition laboratory manual for the examination of human semen, Pg. 16 (pH) and 107 (Leukocytes), Cambridge University Press.



APPENDIX 8: Dilution Media



PRODUCT INSERT

INTRODUCTION AND INTENDED USE:

The QwikCheck™ Dilution kit is used to dilute semen prior to automated or manual semen testing, when indicated. It is also used for sample preparation. The dilution media is Earle’s balanced salt solution which contains ingredients known to support sperm motility and viability. It is recommended by WHO for semen sample preparation (WHO 2010, 5th edition manual, page 163). The product is intended for in vitro use only.

KIT CONTENTS:

- 50 ml of sterile Earle’s Balanced Salt solution / Product Insert

STABILITY AND STORAGE CONDITIONS:

- The product has a one year shelf life. Note the expiration date on the box and bottle
- Store unopened bottle at room temperature. Refrigerate at +4°C after opening.
- Bring to room temperature (22-26 °C) prior to use to prevent cold shock.
- Do not use if the solution contains precipitate or is cloudy.

INSTRUCTIONS FOR USE:

AUTOMATED SQA-V:

1. Measure the volume of the neat semen sample.
2. If the volume is less than 0.5ml, dilute 1:1.
3. Open the dilution kit bottle and pipette the amount of solution equal to the sample volume measured in step 1.
4. Add the solution to the neat semen and thoroughly mix with gentle circular motion, without introducing air bubbles.
5. Fill the SQA-V testing capillary immediately following the directions in the SQA-V user manual.

MANUAL:

1. Follow the laboratory sperm preparation protocols for diluting semen samples for testing.

CLINICAL PERFORMANCE OF SEMEN SAMPLES DILUTED WITH EARLE’S BUFFER (DILUTION KIT CONTENTS):

Parameter:	Correlation Coefficients: Comparison Neat vs. Diluted	Conclusions
Concentration	R = 0.99	Test results demonstrated high correlations for Concentration, Motility, Progressive Motility and Morphology between neat semen samples and those same samples diluted with Earle’s buffer (QwikCheck-Dilution kit) when run on the SQA-V.
Motility	R = 0.84	
Progressive Motility	R = 0.96	
Morphology	R = 0.96	

PRECAUTIONS AND WARNINGS:

Exercise appropriate precautions to minimize direct contact with skin or eyes and prevent inhalation.

REFERENCES:

WHO Laboratory Manual for the Examination of Human Semen, 5th Edition, Cambridge University Press, 2010.

APPENDIX 9: Treating Viscous Samples



INTRODUCTION AND INTENDED USE

The QwikCheck™ Liquefaction Kit can be used to accelerate the liquefaction of viscous semen samples that remain viscous thirty minutes after collection. High viscosity can impact the accurate measurement of motility and concentration. Use QwikCheck™ Liquefaction to prepare viscous semen samples for automated or manual semen analysis. For in-vitro use only.

KIT CONTENTS

- 20 single dose, 5 mg vials of lyophilized α-Chymotrypsin and a product insert.

STABILITY AND STORAGE CONDITIONS

- The product has a one year shelf life. Note the expiration date on the box and vials.
- Vials can be stored at room temperature.

INSTRUCTIONS FOR USE

1. Select one vial of α-Chymotrypsin.
2. Tap the vial to move the contents to the bottom of the vial prior to opening.
3. Add the entire contents of one vial to a viscous semen sample.
4. Gently mix the sample to dissolve the powder.
5. Once the sample has liquefied (5-10 minutes), immediately perform automated testing or neutralize the enzymatic activity (optional) by adding of Human Serum Albumin (HSA) (not provided in this kit).

CLINICAL PERFORMANCE: Semen Samples Treated with QwikCheck Liquefaction:

Parameter:	Correlation Coefficients: Semen samples Treated with Chymotrypsin vs. Non- treated semen samples	Conclusions:
Concentration	R = 0.98	<ul style="list-style-type: none"> • Test results demonstrated high correlations for Concentration, Total Motility, Progressive Motility and Morphology between the treated with chymotrypsin (QwikCheck Liquefaction Kit) and non-treated semen samples when run on the SQA-V. • No detrimental effect is seen when treating semen samples with QwikCheck™ Liquefaction kit containing 5 mg chymotrypsin.
Total Motility	R = 0.99	
Progressive Motility	R = 0.99	
Morphology	R = 0.95	

PRECAUTIONS AND WARNINGS

Each vial contains α-Chymotrypsin, a protease. This protease can cause irritation to eyes, respiratory system or skin. In case of contact with eyes, rinse immediately with water and seek medical attention. Observe the following precautions when handling the product:

- Wear suitable protective clothing: Mask, gloves and laboratory coat.
- Avoid dispersing material over the working area.

REFERENCES: WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction, 5th Edition, Cambridge University Press, 2012.

APPENDIX 10: Assayed Control: QwikCheck™ Beads



A QUALITY CONTROL MATERIAL FOR AUTOMATED AND MANUAL SPERM COUNTING SYSTEMS

Introduction and Intended Use:

QwikCheck™ beads is an in-vitro use only external quality control material for automated and manual sperm counting systems. It has been developed as a tool to assess the accuracy and precision of the laboratory's sperm counting methods by providing a known target value and +/- range. The beads were developed for use on the SQA semen analyzer however, they are also labeled for manual proficiency testing and calibration on hemacytometers such as Neubauer counting chambers, Makler chambers and conventional fixed coverslip chambers.

QwikCheck™ beads is supplied in a kit containing known concentrations of 4-micron latex beads suspended in an aqueous solvent and negative concentration/motility control. The beads should be run according to the directions below for the type of system used in the lab. According to the CLIA '88 regulations, "...for most moderately complex tests, the general requirement is to analyze two levels of QC materials on each day of testing." It is recommended that QwikCheck beads are run on both the SQA automated and visualization systems prior to each day of semen analysis testing.

For in-vitro use only:

Each kit contains two known concentrations of QwikCheck™ beads in two 5 ml aliquots and one 5 ml negative concentration and motility control. Store the beads at room temperature (20-25°C or 65-77°F). The expiration date assumes that QwikCheck™ beads are stored at room temperature in their original containers and tightly capped to prevent evaporation. Open vial shelf-life is 90 days (tightly capped, stored in the original container at room temperature or refrigerated @ 5-15 degrees Centigrade). QwikCheck™ beads are stable and show no loss of expected performance characteristics after transport/storage over a period of 72 hours at the temperature range of -20°C to +37°C.

Target Value and +/- Ranges

Target Values and +/- Ranges unique to the batch have established for each batch of QwikCheck beads. Each box and each control bottle is labeled with these Target Values and Ranges. In addition, the MES website: www.mes-global.com provides Batch Release Forms by batch # with details.

Warning:

Contains 0.1% Sodium Azide as a preservative. Other ingredients are not harmful due to the low concentration in the beads. For additional information, please refer to the QwikCheck-beads Safety Data Sheet # QCB 001

Basic Instructions for using QwikCheck™ beads

1. BEFORE initially opening new beads, turn the bottle upside down and gently shake. During shipment some beads adhere to the top of the bottle.
2. Mix the beads in the closed container by gently rotating by hand. This will evenly distribute the beads in the suspension and insure accurate test results. Do not use a vortex except for the Neubauer chamber which requires vortexing because the beads are diluted with water.
3. Open the container and immediately withdraw a sample of the control material. Tightly close the container after withdrawing the sample.
4. Follow the detailed instructions below for the type of system/chamber used.

Instructions for running QwikCheck™ beads QC material on automated and manual sperm counting systems:

SQA Automated System:

1. Refer to the SQA User Guide "CONTROLS" section for an explanation of how to set-up the SQA to test automated Level 1/Level 2 and Negative Control QwikCheck™ beads. Follow the SQA onscreen instructions in the "Controls" section of the SQA User Guide:
2. Before opening new beads, gently rotate the closed container of beads upside down to remove any beads that may have adhered to the top liner.
3. Mix opened beads by gently rotating the close container (do not use a vortex).
4. Aspirate a sample of the beads or negative control into the SQA capillary in the same manner you would fill the capillary for a normal volume specimen. Make sure that the cuvette section of the SQA capillary is completely full of liquid and free of bubbles.
5. Insert the testing capillary into the SQA measurement chamber when prompted.
6. Use a new capillary for running each level of beads.

SQA Visualization System using a standard slide:

1. Refer to the SQA / VISION User Guide for instructions how to use slide in the SQA visualization.
2. Gently rotate the closed container of beads by hand (do not use a vortex).
3. Pipette 10 µL of QwikCheck™ beads onto a standard slide, cover with a 22x22 mm coverslip to provide a 20-micron sample depth.
4. If liquid spills onto the slide or air bubbles are seen, prepare a new slide to ensure accurate results.
5. Insert the slide into the slide adaptor and then into the visualization chamber of the SQA. Press Zoom-Out all the way to set the magnification at x300 and FREEZE the image.
6. Count the beads manually per WHO guidelines: Duplicate counts of at least 200 beads are required (turn the slide adaptor knob to view multiple fields). Divide the final number of beads counted in the multiple fields by the number of screens viewed. Each bead on the SQA / VISION screen represents 1 M/ml.
7. Refer to the WHO Manual 5th Edition Manual, table 2.4 to determine if the duplicate counts are acceptable.

Appendix 11: Concentration Standard: Counting Chambers

A number of commercially available counting chambers are used in laboratories for manually counting sperm cells. These chambers vary by depth and one type requires a diluted sample. It has been clinically established that counts vary by approximately 30% depending on the chamber used.

The SQA-V permits the user to select the type of chamber the laboratory has implemented as a standard for manual semen analysis. Once the concentration standard (CONC. STANDARD) has been selected the SQA-V will automatically run semen samples based on that standard.

SQA-V Set-Up:

- Select SERVICE > SERVICE DATA.
- Log into V-Sperm and go to **SET-UP > SQA-V > CONTINUE**
- Select a **CONC. (concentration) STANDARD** based on aligning the system with the options shown in the table below:
 - **CONC. STANDARD #1**
 - **CONC. STANDARD #2**
- Commercially available counting chambers are divided into two unique groups:
- **Standard #1:** 10-20 micron depth and do not require sample dilution.
- **Standard #2:** 100 micron depth (haemocytometers) that require sample dilution.

The table below classifies some commercially available chambers:

CHAMBER STANDARD #1	CHAMBER STANDARD #2
Makler	Beurker-Tuek
Micro-Cell	Buerker
Fixed Cover slip disposable chambers	Fuchs-Rosenthal
	Fuchs-Rosenthal (modified)
	Improved Neubauer
	Neubauer
	Malassez
	Thoma
	Thoma Modified

Appendix 12: Post-vasectomy Protocol

The SQA-V runs a five minute POSTVASECTOMY test that can detect the presence of a very small number of motile cells. Once the automated test has been performed the user is given the option to follow the POSTVASECTOMY protocol outlined below (also refer to the Appendix section of this guide) and "scan" the testing capillary in the SQA-V visualization system.

By scanning through the depth of the testing capillary the user is able to identify and readily count immotile cells and visually confirm automated test results. Clinical studies positively demonstrated that by incorporating both the SQA-V automated AND the visualization system in the testing protocol, a very high level of accuracy is obtained for identifying motile and non-motile cells in POSTVASECTOMY samples.

In order to obtain similar levels of accuracy it is imperative that the user strictly follow the manufacturer's protocol outlined below. Additionally, once the testing cycle is complete, the user has an opportunity to document test results by capturing and archiving a video clip of the postvasectomy specimen using V-Sperm™ software.

This test is highly sensitive to any movement. The SQA-V and the testing capillary should not be disturbed in any way during the 5 minute testing cycle.

- Fill BOTH sections of the SQA-V testing capillary (for stability during the 5-minute test).
- If the specimen volume is not adequate to fill both sections, dilute it with Earle's Buffer and multiply results by the dilution factor.
- Follow the user guide for instructions on running a POSTVASECTOMY sample.
- Run the automated 5-minute test for motility parameters.
- Remove the capillary and insert it into the visualization system and "scan" ten fields of the SQA-V capillary following the user guide instructions.
- Enter the number of motile and immotile sperm cells visualized.
- The final test results will report the greater number of cells found in the automated or visualization test.
- Leave the testing capillary in the visualization system.
- Save the test to the SQA-V archive and import it to the V-Sperm GOLD software.
- Following the V-Sperm user guide instructions, import the test into the V-Sperm data base and attach a live VIDEO clip to the patient's test record for documentation purposes.
- *NOTE: If the SQA-V is reporting > 30 motile spermatozoa, a screen will indicate that a NORMAL TEST should be run instead of POSTVASECTOMY > 30 motile spermatozoa is equivalent to MSC > 2M/ml.*

APPENDIX 13: GLOBOZOOSPERMIC SAMPLES

ASSESSING GLOBOZOOSPERMIC SAMPLES ON THE SQA-V (VERSIONS: All Human SQA-V GOLD Systems)

BACKGROUND: The absence of an acrosome in the head of the sperm cell (globozoospermia) cannot be assessed automatically by the morphology test of the SQA-V. This technical bulletin describes the incidence of this disorder and demonstrates how to identify these samples prior to running the automated SQA-V semen analysis.

WHAT IS THE INCIDENCE OF GLOBOZOOSPERMIA? An article in Human Reproduction (January/February 2007) 13 (1): 63-75 **GLOBOZOOSPERMIA REVISITED** best describes this condition and incidence:

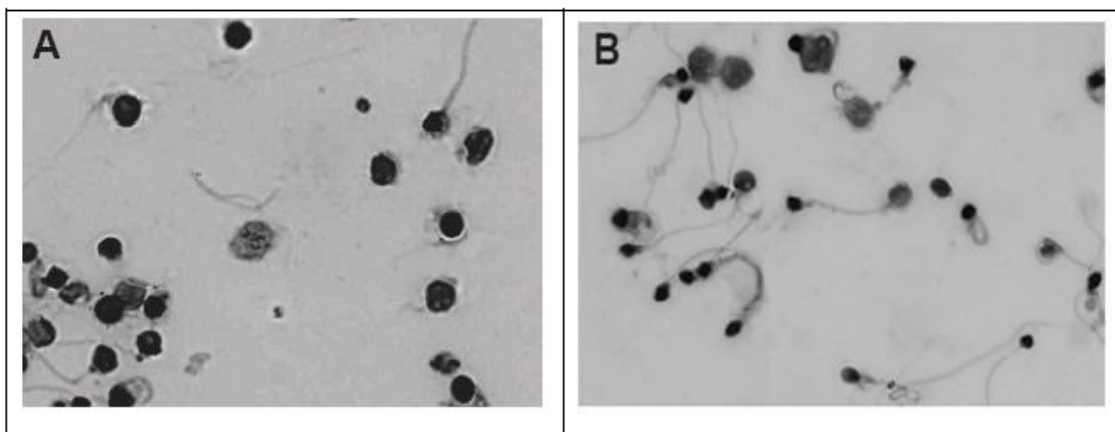
Abstract

Globozoospermia is a rare (incidence <0.1%) but severe disorder in male infertility. Total globozoospermia is diagnosed by the presence of 100% round-headed spermatozoa lacking an acrosome. It is still unclear whether patients whose ejaculate contains both normal and globozoospermic cells (partial globozoospermia) suffer from a variation of the same syndrome. Apart from the fact that affected males suffer from reduced fertility or even infertility, no other physical characteristics can be associated with the syndrome. ICSI is a treatment option for these patients, although low fertilization rates after ICSI show a reduced ability to activate the oocyte. In globozoospermic cells, the use of acrosome markers has demonstrated an absent or severely malformed acrosome.... The pathogenesis of globozoospermia most probably originates in spermiogenesis, more specifically in acrosome formation and sperm head elongation...More research is needed to elucidate the pathogenesis of human globozoospermia to further understand globozoospermia as well as (abnormalities in) spermiogenesis and spermatogenesis in general. Globozoospermia is normally diagnosed by the detection of round-headed sperm heads during routine light microscopic examination of a semen sample.

For the full article please go to: <http://humupd.oxfordjournals.org/content/13/1/63.full>

SCREENING FOR GLOBOZOOSPERMIA when using the SQA-V:

“Globozoospermia is normally diagnosed by the detection of round-headed sperm heads during routine light microscopic examination of a semen sample.” Before running samples on the SQA-V, prepare a standard slide and view it in the visualization system of the SQA-V to screen for globozoospermia. Some examples of globozoospermia are demonstrated below.



APPENDIX 14: SQA-V SERVICE SUPPORT REPORT

**SQA-V SERVICE SUPPORT
Parameter Report**

Device number: _____ SQA-V Software Version: _____ Date: _____

Instruct the user to run a SERVICE report. For version 2.60 from the MAIN MENU select: **SERVICE > PRINT SELF TEST DATA.**

Calibration parameters:

Fill-in the USER REPORT column with the calibration parameters found in the INTERNAL DATA SECTION of the SERVICE DATA REPORT run on the "defective" SQA-V. Contact MES for the initial calibration parameters. These parameters should not have changed.

Parameter	Service Report Item #	User Report	MES Report	Comments
CONTR.REF1	#1			
OD AMPLIF.	#13			
MSC AMPLIF	#8			
OD VALUE	#15			
OD CORR	#16			
LB OD AMP	#18			
CONTR. Z.L*	#11			

*CONTR. Z.L. can be adjusted in the field by a MES trained service technician.

Algorithm parameters:

Fill-in the User Report values for the following algorithm parameters found in the SERVICE DATA REPORT. The SQA-V algorithm settings are defined and should not have changed.

Parameter	Service Report Item #	User Report	MES Settings	Comments
MIN.SP.HEIGHT	#2		5	
MIN.SP.WIDTH	#9		10	
MAX.SP.WIDTH	#3		150	
NOISE THRESH	#10		6	
SMI THRESH	#4		28	

Self Test Parameters:

Fill-in the SQA-V SELF TEST PARAMETERS from the SELF TEST printout in V-Sperm:

- The SQA-V must be connected to the PC and V-Sperm activated.
- From the **SERVICE>SERVICE DATA** screen of the SQA-V:
 - Go to the V-Sperm navigation buttons: **UTILITIES>SELF TEST DATA**
 - Select **PRINT**
 - Verify that the parameters listed below fall within the established range
 - Highlight the discrepancies and report to MES

<i>Parameter</i>	S/W Ver. 2.60 Criteria	SQA-V Self-Test Parameters	
Ref. 1	150 – 400 mV		
LED Current 1	5 – 25 mA		Original value
Amplitude	50 – 100 mV		
Count (#12)	26 – 36		
Zero Level	500 – 525		
Ref. 2	2500 – 3500		
LED Current 2	10 – 32 mA		Original value
TSC 1 or CONC 1	0 – 1 M/ml		
TSC 2 or CONC 2	50 – 150 M/ml		
TSC 3 or CONC 3	300 – 600 M/ml		

APPENDIX 15: SQA-V Reports

Semen Analysis Report

```

*****
SEMEN ANALYS. REPORT
*****

SQA-V SN      992
SW VER.      02.60.15
TEST DATE    15/07/13
TEST TIME    10:32
PRINT DATE   15/07/13
PRINT TIME   11:44

PATIENT DATA

ID            4
BIRTH DATE   11/11/11
ABSTINENCE   4 DAYS

SAMPLE DATA

ACCESSION #*  5
COLLECTED     12/12/11
              10:31
RECEIVED      10/05/13
              15:45
TYPE          WASHED
VOLUME        7ml
WBC CONC.    < 1M/ml
PH            4
APPEARANCE    NORM.
VISCOSITY     NORM.
LIQUEFACTION ABNORM.

TEST RESULTS

CONC.         < 2.0M/ml
TOTAL MOTILITY
<PR+NP>       < 17%
MOTILITY GRADES:
PRG. <PR>     %
NONPRG. <NP>  %
IMMOT. <IM>   %
MORPH. NORM. FORMS
<WHO 5th>     %
MSC           < 0.2M/ml
PMSC         M/ml
FSC          M/ml
VELOCITY     mic/sec
SMI          3

TOTALS PER VOLUME

SPERM #       N.A.M
MOTILE SPERM  N.A.M
PRG. SPERM    N.A.M
FUNC. SPERM   N.A.M
MORPH. NORM. SPERM
SPERM         N.A.M

SYSTEM DATA
S.  0.00  T.  0.000  12.  4
Ml  250   19.  2   Conc.Std.  2
    
```

Service Data Report

```

SERVICE DATA
REPORT

SQA-V SN      83
SW VER.      02.60.04
PRINT DATE   02/08/11
PRINT TIME   10:49

SELF-TEST DATA

REF. 1        370mV
LED CUR. 1    19mA
AMPLITUDE     68mV
SMI           33
ZERO LEVEL    514
REF. 2        2930mV
LED CUR. 2    30mA
CONC. 1       0.0M/ml
CONC. 2       102.8M/ml
CONC. 3       418.9M/ml

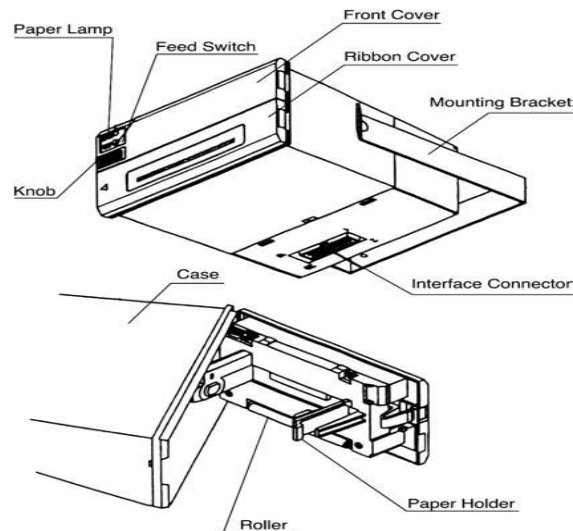
ALGORITHM

MSC           40.6M/ml
CONC.         418.9M/ml
N. MORPH.     3%
VELOCITY      4mic/sec
NUMBER SPIKE  61
AREA          40.84
MOTILITY      10%
FSC           1.4M/ml
AVG. WIDTH    15076

SERVICE DATA

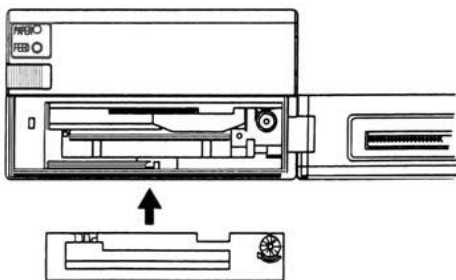
1.           30
2.           5
3.           150
4.           10
5.           40.84
6.           514
7.           0.000
8.           60
9.           10
10.          8
11.          120
12.          31
13.          216
14.          100
15.          1.70
16.          100
17.          3
18.          1000
19.          1
    
```

APPENDIX 16: Printer Ribbon/Paper Installation



Installing the Printer Ribbon:

1. Turn off the power and open the Ribbon Cover – remove old ribbon cassette
2. Cut away any paper that is obstructing the ribbon installation area
3. Confirm that the new ribbon cassette is placed in the correct way (see below)
4. Insert the new ribbon between the printing head and the platen – press the cassette down from the knob side
5. Remove the ribbon slack by turning the ribbon in the correct direction to tighten it

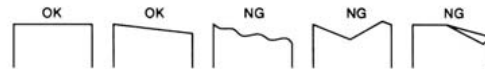


PLEASE NOTE:

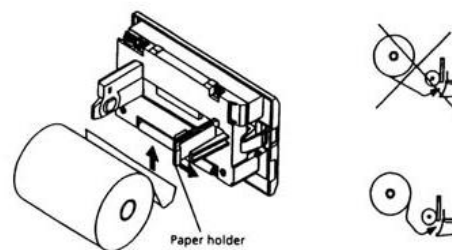
- Use only M.E.S. supplied ribbons
- Do not print if there is no ribbon in the holder
- Ribbons will dry out if sitting for a long time in the printer

Installing Printer Paper:

1. Open the front cover to expose the paper holder and roller
2. Cut the edge of the paper as shown below



3. Insert the paper into the printer mechanism as shown below. The paper will automatically advance OR press the FEED SWITCH to advance the paper (advance one line at a time by pressing once; press and hold to feed continuously)
4. Load the paper roll into the brackets – make sure the paper roll is feeding paper in the correct direction – see example below.



PLEASE NOTE:

- Load the paper in the direction shown above
- Use only M.E.S. supplied paper – standard rolls are too large for this printer and will damage it
- Do not print when there is no paper or during loading
- Do not pull on the paper in the reverse direction
- Paper will jam if fed diagonally or incorrectly, in this case turn the printer OFF and gently pull the paper in the right direction

APPENDIX 17: Warranty



Sperm Quality Analyzer

SQA, QwikCheck™ GOLD

Warranty

Medical Electronic Systems ("MES") warrants that the Sperm Quality Analyzer will be free from defects in workmanship and materials for a period of twelve (12) months from date of purchase. During the warranty period, if the device is shown to MES's reasonable satisfaction to be defective, MES shall, at its option, repair such a device without charge for parts or labor. The foregoing remedy shall be purchaser's sole and exclusive remedy under this warranty. In the event (i) purchaser makes any modifications or alterations to the SQA /QwikCheck GOLD or (ii) the SQA/QwikCheck GOLD is used, operated, opened or serviced other than as directed by MES or is damaged as a result of use, careless transportation (not in its original box, or within the allowed temperature range, operation or servicing other than as directed by MES, the foregoing warranties shall be void and of no further force or effect. EXCEPT FOR THE FOREGOING WARRANTIES, THE PRODUCTS ARE SOLD AS-IS AND WITHOUT ANY OTHER WARRANTY OF ANY NATURE WHATSOEVER. MES HAS NOT MADE AND DOES NOT MAKE ANY OTHER REPRESENTATION, WARRANTY, GUARANTY, OR COVENANT, EXPRESS OR IMPLIED, WITH RESPECT TO THE DESIGN, CONDITION, DURABILITY, SUITABILITY, FITNESS FOR USE, FITNESS FOR A PARTICULAR PURPOSE, OR MERCHANTABILITY OF THE SQA IN ANY RESPECT. UNDER NO CIRCUMSTANCES AND IN NO EVENT, WHETHER AS A RESULT OF BREACH OF CONTRACT OR WARRANTY, TORT (INCLUDING NEGLIGENCE AND STRICT LIABILITY) OR OTHERWISE, INCLUDING BUT NOT LIMITED TO INACCURATE RESULTS OR OPERATOR ERROR, SHALL MES BE LIABLE FOR ANY SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES. IN NO EVENT SHALL MES'S LIABILITY WITH RESPECT TO THE PRODUCT EXCEED THE PURCHASE PRICE FOR SUCH PRODUCT.

**Extended service contracts are available for purchase.
Please contact the dealer or supplier for information.**

Serial Number: _____ Date Purchased: _____
Dealer: _____ Dealer Phone#: _____
Purchaser: _____ Purchaser Phone #: _____

APPENDIX 18: Product Performance Data

Abbreviations:

TSC: Sperm Concentration (Count) MSC: Motile Sperm Concentration
 PMSC: Progressive Motile Sperm Concentration Morph Norm Forms: Morphologically Normal Forms
 OD: Optical Density MV: Millivolt

Performance Data Summary

The performance the SQA-V is summarized in the text, tables and graphs below. All values concerning sperm concentration measurements are expressed as x10⁶ sperm cells per milliliter (M/ml). Motility and Morphology values are expressed as a percent (%). All testing was performed using human patient and donor semen samples.

Calibration:

Each SQA-V is biologically calibrated against two reference systems at Medical Electronic System's laboratory.

Dynamic Range:

Sample Type	Test Mode	Sperm Conc. M/ml	Motility %	Morph %	MSC M/ml	PMSC M/ml	#Sperm Cells/field
Fresh	Normal	<2-400	0-100	2-30	<.2-400	0-400	-
Washed	Normal	<2-200+	0-100	2-30	<.2-200+	0-200+	-
Frozen	Normal	-	-	-	<.2-200+	0-200+	-
Post-Vasectomy		-	-	-	0-2	-	0-30

Precision

Precision and Accuracy Established Against a Known Target (Latex beads)

Background: The precision and accuracy of the SQA-V was compared to a known target value using latex beads (Accu-beads®).

Latex beads are used as a quality control product to validate the accuracy of sperm counting methods for two known levels of concentration. In accordance with CLIA regulations such a control is used to demonstrate operator proficiency using the microscope and for validation of automated sperm counting methods. The latex beads were run in 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

Method comparison:

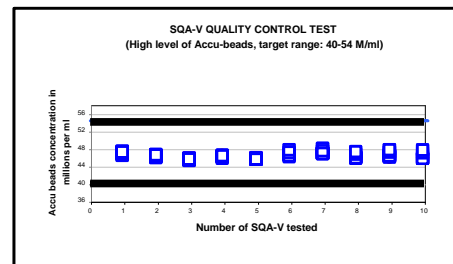
A total of 320 latex bead samples were tested on ten SQA-V systems (32 samples/SQA-V). SQA-V concentration readings were compared to established target values +/- acceptable range.

Latex beads established target values +/- ranges (Hemocytometer):

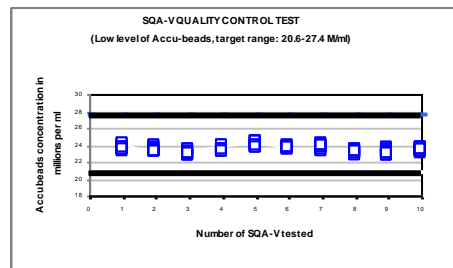
- Vial #1: 47 +/- 7.0 M/ml
- Vial #2: 24 +/- 3.4 M/ml

SQA-V	Accu-beads®	CV, %
Intra-device Variability	High 47 ± 7.0 M/ml	≤ 0.01
	Low 24 ± 3.4 M/ml	≤ 0.01
Inter-device Variability	High 47 ± 7.0 M/ml	≤ 2.00
	Low 24 ± 3.4 M/ml	≤ 2.50

Accuracy: High Level Control



Accuracy: Low Level Control



Precision and accuracy established in clinical trials using human semen samples

Clinical claims:

Specificity

- Concentration: 85%
- Motility: 80%
- Morph. Norm Forms (WHO 3rd): 65%
- Morph. Norm Forms (WHO 4th): 60%
- Morph. Norm Forms (WHO 5th): 90%
- Postvasectomy: 90% of motile cells detected

Sensitivity

- Concentration: 90%
- Motility: 85%
- Morph. Norm Forms (WHO 3rd): 85%
- Morph. Norm Forms (WHO 4th): 65%

Correlation to Manual Method

- Concentration: 0.9
- Motility: 0.85
- Morph. Norm Forms (WHO 3rd): 0.65
- Morph. Norm Forms (WHO 4th): 0.45
- Morph. Norm Forms (WHO 5th): 0.45

Linearity

Linear Sperm Concentration throughout the SQA-V dynamic range of 2M/ml to 400M/ml

- Squared regression coefficient of Dilution Curve $R^2 \geq 0.9$.
- Averaged coefficient of variation CV of measured vs. expected sperm concentration $\leq 20\%$.

Note: Claims are less than actual correlations noted (see tables 1 and 2).

Background: The SQA-V concentration, motility and morphology readings were compared to standard microscopic results based on WHO 3rd, 4th and 5th standards and MES protocols. Four independent clinical trials were conducted at MES lab, Tel Hashomer andrology dept and Ramat Marpe lab (Israel) and ART laboratory, University Hospital of Nantes (France). A total of >750 human semen samples were analyzed as described below with approximately 350 samples of low quality and tested in the Postvasectomy mode. Among them, 246 semen samples were tested at University Hospital of Nantes.

#Samples	Fresh	Washed	Frozen	High Sensitivity
>750	>300	42	30	>350

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 clinical specificity:

- Highly viscous samples can only be read accurately with liquefaction (QwikCheck™ Liquefaction Kit used).
- Sample size must be >0.7ml for fully automated tests.
- % Normal Morphology is a parameter derived from the electronic signals of the system by a proprietary algorithm. This is not a direct assessment of the stained smears.
- Results obtained from the use of the SQA-V visualization system may be affected by the subjectivity of the operator.
- Dynamic range limitation as stated above.

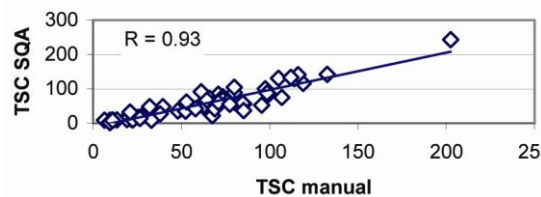
Table 1: Sensitivity/Specificity		
SQA-V vs. Microscope	Sensitivity	Specificity
Trial #1:		
Concentration	100%	95%
Motility	97%	85%
Morph Norm Forms (WHO 3 rd)	94%	75%
Trial #2:		
Concentration	94%	90%
Motility	87%	90%
Morph Norm Forms (WHO 4 th)	69%	70%
Trial #3: High Sensitivity (Postvasectomy - see table #5)		
Motile Sperm Cells	95%	95%
Immotile Sperm Cells	99%	100%
Trial #4 (ART laboratory, University Hospital of Nantes, France):		
SQA-V vs. Microscope	Negative Predictive Value	Specificity
Morph Norm Forms (WHO 5 th)	92.5	97.9

Table #2: Correlation to Manual Method

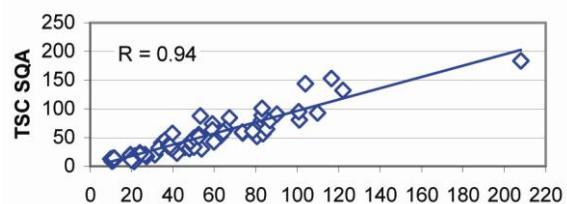
Parameters	Correlation Coefficients	
	Trial #1	Trial #2
Sperm Concentration	0.93	0.94
Motility	0.86	0.87
Morphology WHO 3 rd	0.66	-
Morphology WHO 4 th / 5 th	-	0.49*
MSC	-	0.79

* Correlation is low due to narrow dynamic range of this parameter per strict criteria and manual analysis subjectivity.

1st clinical trial - TSC correlation



2nd clinical trial- TSC correlation



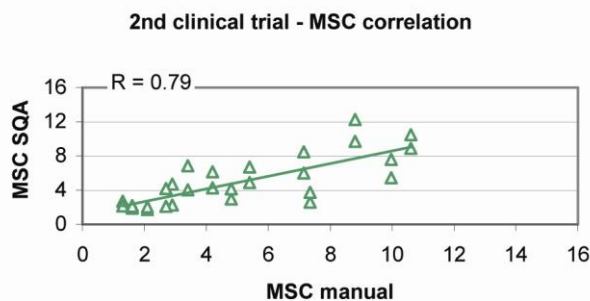
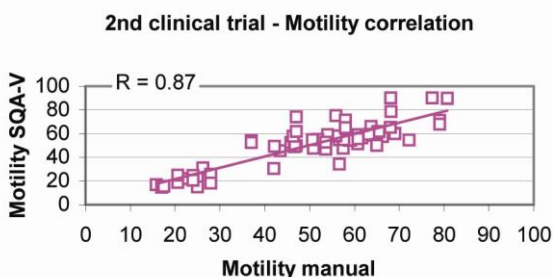
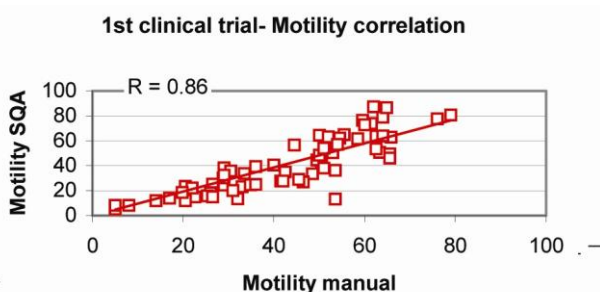
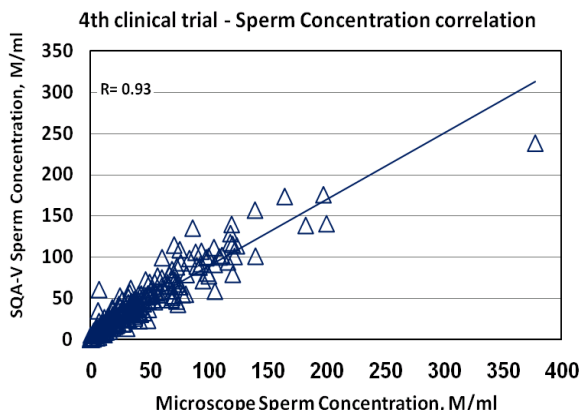
Method comparison:

- SQA-V was compared to the microscope based on WHO 3rd (Trial #1), 4th (Trial #2) and WHO 5th (Trial #4) guidelines.
- **Sensitivity and Specificity** were calculated using ROC curves with the cutoffs based on the reference values of WHO 3rd, 4th and 5th guidelines (see Table #1).
- **Correlation** coefficients of the SQA-V results to the manual method are presented in the Table #2.
- **Precision:** Inter-device (Tables #3) and intra-device (Table #4) variations were compared to inter- and intra-operator variability using Coefficients of Variation (CV, %). Duplicate samples were assessed by two methods. The CVs characterizing precision were calculated for multiple semen parameters.
- The **POSTVASECTOMY** test (Trial #3) compared three assessment methods:
 - Microscope (standard slide; X400; 10 fields of view)
 - SQA-V (SQA-V + SQA-V visualization)
 - SQA-V visualization system (see table #2).
- Immotile cells were analyzed by use of the SQA-V visualization system.
- 218 semen specimens contained motile cells and were used as the basis for the Post Vas visualization method comparison (Table #5).

Parameter	Range	Method	
		SQA-V CV%	Microscope CV%
Sperm Concentration	Entire Range	3.1	6.1
	5-40	5.2	5.9
	41-80	2.1	5.5
	>80	2.5	3.2
Motility	Entire Range	5.1	7.2
	10-50	7.6	10.3
	51-55	1.5	3.4
	>55	6.0	4.1

Semen Parameter	Mean		CV, %		
	Op1	Op2	SQA-V	Manual	SQA-V
Sperm Concentration	41.0	40.2	41.4	11.5	3.4
Total Motility	54.7	56.9	54.9	10.7	5.0
PR Motility	37.9	39.0	36.6	13.3	7.5
NP Motility	16.8	17.9	18.4	27.3	6.8
Morphology	7.6	7.6	11.5	27.4	6.5

Note: Op1 - operator 1; Op2 - operator 2



Limitations of method:

Samples were assessed by different operators using a microscope and the SQA-V. Inter-operator subjectivity may have affected the results of the study.

Table #5: Percentage Motile Cells Detected: Trial #3 Postvasectomy mode

Method Comparison of 218 Samples with Motile Cells	# Samples Motile Sperm Detected	% Samples Motile Sperm Detected
SQA-V Automated System and Visualization System	207	95%
Visualization System only	193	89%
Microscope only	161	74%

SQA-V Linearity

Clinical claims:

- Linear Sperm Concentration throughout the SQA-V dynamic range of 2M/ml to 400M/ml:
 - Squared regression coefficient of Dilution Curve $R^2 \geq 0.9$.
 - Averaged coefficient of variation CV of measured vs. expected sperm concentration $\leq 20\%$.

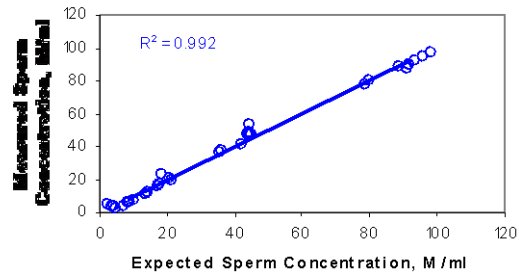
Goal: To demonstrate the ability of the SQA-V to accurately report sperm concentration along the dynamic range of the system using sequentially diluted human semen samples.

Methodology: 4 fresh human semen samples were pooled, divided into two aliquots and centrifuged at 600g for 15 minutes. The seminal plasma was decanted and the pellets were re-suspended in washing media: DPBS & HepesHTF. Sequential dilutions were run in 4 SQA-V systems.

Limitations of method:

- Dilution errors contribute to the accuracy of the linearity test results.
- Sample handling errors such as the introduction of bubbles into the testing capillary can cause inaccurate readings.

SQA-V DILUTION CURVE USING SEMEN DILUTED WITH DPBS & HEPES SOLUTION



Results:

1. Squared regression coefficient R^2 of Dilution Curve (trend line) was found to be 0.992 (note: graph displaying results of four SQA-V's and DPBS and Hepes dilution media).
2. Averaged coefficient of variation CV of measured vs. expected sperm concentration was 10%.