Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer

Purpose

This procedure provides instructions for performing semen analysis using the SQA-VISION automated sperm quality analyzer mixed technology method.

Scope

This procedure is intended for testing personnel trained in the activities outlined in this procedure.

Specimen

- Specimen Type: Fresh Semen
- Specimen Volume: Entire ejaculate is required for determining sample volume
- Minimum Volume:
  - 0.3 mL(SQA-Vision Auto)
  - 0.1 mL(SQA-Vision Manual)
- Maximum Ejaculation to Test Time: 1 hour

Specimen Collection

Provide the patient with local instructions for semen collection, and verify that they have followed these instructions summarized below:

- 2-7 days abstinence from ejaculation prior to specimen collection
- Collect sample by masturbation or by special direction from physician
- Lubricants, spermicides and other contaminants are not to be used.
- The entire specimen must be collected into a clean container supplied only by the provider's office or laboratory.
- The specimen container should be clearly labeled with the patient's first and last name, medical record number, and date and time of collection.
- Keep specimen at room temperature. DO NOT refrigerate or expose to heat.

Specimen Transport and Temperature

- Transport the specimen to the laboratory right after collection (within 60 minutes after collection) for an accurate evaluation of sperm motility.
- During transport to the laboratory, the sample should be kept between 20 °C and 37 °C.
- Do not heat or cool the sample nor the container

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Specimen Stability

- The semen sample must be tested within one hour of collection because motility will decline.
- Semen samples must be tested by the laboratory on a priority basis upon delivery, and expedited to the testing area.

Specimen Handling Prior to Testing

- When a patient arrives at the laboratory with his specimen, he is given the Patient Questionnaire Form by the receiving laboratory personnel to fill out. See **Procedure for Managing the Semen Analysis – Patient Questionnaire Form** and **Semen Analysis – Patient Questionnaire Form**.
  Important Note: Use the information in the completed Patient Questionnaire Form to result in Cerner.
- The collection container should remain at room temperature until liquefaction is complete or 45 minutes, whichever is shorter.
  - Some samples will not liquefy within 45 minutes (most will liquefy within 15 minutes).
  - If a specimen is not liquefied, the accuracy of the analysis will be compromised.

Specimen Rejection

- The following rejection criteria are recommended by the vendor/manufacturer.
  - If testing is greater than 60 minutes but less than 2 hours after sample collection, results are questionable due to age of specimen.
  - If testing is greater than 2 hours after collection, reject the specimen.
- See procedure block ***Cerner Resulting*** to report the required **Analysis Time** and **Analysis Time Comment** in Cerner.

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Equipment, Reagents, Materials and Supplies

- SQA-VISION Analyzer and V-Sperm Software
- QwikCheck Liquefaction Kit (Catalog #0900)
- QwikCheck Beads (Catalog #0200)
- QwikCheck Test Strips for Semen Analysis (Catalog #0700) using BioRad Urinalysis Controls
- QwikCheck Dilution Kit (Catalog #0800)
- SQA-V Capillaries (Catalog #0402)
- SQA-V Cleaning Kit (Catalog #0115)
- QwikCheck Fixed Cover Slip Slides Kit (Catalog # A-CA-01082-00) Medical Electronic Systems, LLC
- Microscope Slides, Glass, 1” x 3”
- Coverslips, 22 x 22 mm
- pH Indicator Paper
- Vortex Mixer
- Dilution Container
- Timer
- Thermometer with Humidity Sensor

Workplace Safety

Refer to your local procedure(s) for workplace safety.

Preventive Maintenance

- Perform daily and weekly maintenance as described in the Daily Maintenance and Inspection for the SQA-VISION Sperm Quality Analyzer provided by SCPMG Laboratory Technology Services.
- When to Clean:
    - Daily when running samples
    - Weekly
    - After every 10-15 tests
    - After ANY spillage
    - If Self-test or any failure occurs
    - If system becomes contaminated with semen
- **ONLY use the Manufacturer’s cleaning kit and cleaning brush or damage will occur to the SQA-VISION film and the system will not operate!**

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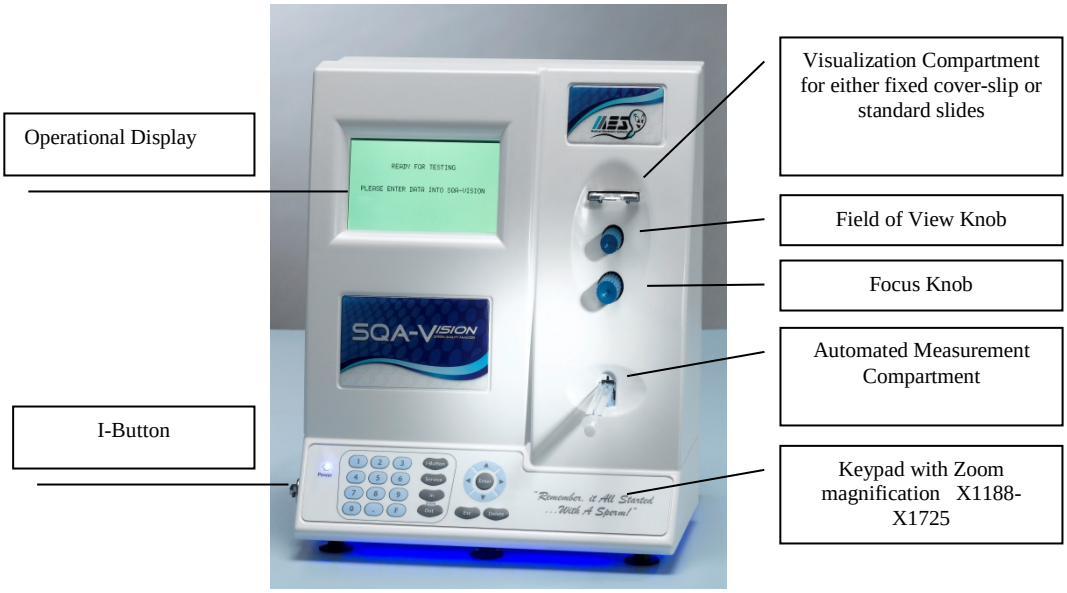
Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Preventive Maintenance, continued

Manufacturer's Recommendations:

- Operate the SQA-VISION away from devices that may cause electronic noise or other devices causing vibrations such as centrifuges.
- Turn the SQA-VISION analyzer OFF at the end of the day and leave OFF when not in use for extended period of time.
- Maximum operational humidity is up to 80% for temperatures of up to 31°C with decreasing linearly to 50% at 38°C.
- The system 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). Have a thermometer available for temperature monitoring near the testing area. Note: Extreme ambient temperature may impact the accuracy of motility test results because of the known effect of temperature on human semen.

FRONT PANEL



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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

REAR PANEL

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Add I-Button

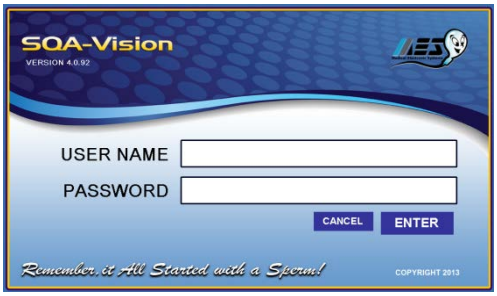
Select **ADD TESTS TO COUNTER** from the **SERVICE MENU** or press the **I-Button** key to open the screen below and follow instructions:

Continued on next page

Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Start up and Auto Calibration

Follow the steps below to perform start up and auto calibration on the SQA-VISION.

Step	Action
1	Turn on the SQA-VISION by pressing the main switch located on the left side. The SQA-VISION automatically performs a five to seven minutes System stabilization, Auto-Calibration and Self-Test. ***Notes:*** • During this period, do not touch the system, do not insert capillary/slide into the device, and do not use any keyboard functions. • If stabilization or self-test fails, you will receive an error code. See error and warning messages in the SQA-VISION user guide for resolution that includes recalibration and re-stabilization of the system.
2	• The device communication screen will appear when the System Self-Test process is complete as “Ready for Testing. Please Enter Data Into SQA-VISION”.
3	• Turn on the SQA-VISION computer (PC). Located on the PC Desktop, double click the SQA-VISION icon to open the SQA-VISION software. Enter the following:  • USER NAME: **administrator** • PASSWORD: **fertility** ***Note:*** Once logged in, Home Screen will download Service Data Status Control Status, Tests Stats, and Back up Status.

Continued on next page

Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

4	• Once instrument is ready, check instrument settings: Click SETTINGS> TEST PATIENT 1. CONC. STANDARD: **Conc. Standard 2** 2. LES: **ROW**

Quality Control

Follow the steps below to prepare and run quality controls.

Notes:

- Three levels of latex QwikCheck bead controls are run each day of use.
- With each new lot of QwikCheck beads assayed control to be run, the user **must** set-up/update the CONTROL settings by following the SQA-VISION User Guide instructions for updating previous CONTROL settings (defaults).
- Verify that quality control results are within acceptable range before testing patients.
- Two levels of Biorad Urinalysis controls are run each day of use using Qwik Check Strips for WBC and pH.

Step	Action
1	Click the QC/Proficiency tab on the left side of the SQA-VISION window. ***Note:*** Performing QC is done in the Latex Beads tab; this window will show the current Quality Control Data (Status, Lot# in use, Exp. Date, Target and Range).
2	Before testing QC, check that all Service Data Parameters have passed and current QC lot# in use has the correct expiration date, target and date. If all QC information is correct, proceed to **Step 5.** Otherwise, proceed to **Step 3** to update the CONTROL settings.
3	Click **Setup** on the lower right of the window to update QC data for new lot of QwikCheck beads. This will link to Control Settings window.
4	Under Settings >Control Tab, click **barcode** under Latex Beads to scan barcode from the QwikCheck beads QC material box. This step will retrieve all the QC Data necessary for QC testing. ***Note:*** If barcode scanner is not available, enter manually and fill up all the fields in the QC settings data. Click **Save** to keep Settings.

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Quality Control, continued

Step	Action
5	Before opening the control box, verify that the control lot number is the current lot number in use and thoroughly mix the QwikCheck beads in the closed container by gently rotating the beads by hand (do not use a vortex). ***Notes:*** • It is imperative that the beads are evenly mixed without creating bubbles in order to insure accurate results. • The negative control does not require extensive mixing.
6	• Open and aspirate the beads into a clean SQA-V capillary in the same manner you would fill the capillary for a normal volume specimen, making sure the cuvette section of the capillary is completely full of liquid and free of bubbles. Refer to procedure block ***Filling the SQA-V Testing Capillary***. • Immediately and tightly close the control container after withdrawing the sample to avoid evaporation and spillage.
7	Under QC/Proficiency > Latex Beads tab, click **TEST NOW** on the desired level of QC latex beads to be run to open the sample preparation instructions screen.
8	Follow the SQA-VISION on-screen instructions: "Insert the SQA-V capillary into the testing chamber". Testing will begin automatically. ***Notes:*** • Make sure to wipe free of any sample before insertion. • Control test results will be displayed on the SQA-VISION screen, and are automatically saved.

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Quality Control, continued

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>9</td><td>
• The PASS/FAIL results will be displayed based on the test results vs. the target value and +/- range (disregard this for non-assayed controls whose target range is set to “0”).
• A CORRECTIVE ACTION button is displayed for all FAILED results. Click this button to open the table which provides a list of problem descriptions and associated corrective actions.
• Implement a corrective action and re-run the test, or follow the table below if any of the listed corrective action does not apply.
<table>
<tr><th>If quality control result…</th><th>Then…</th></tr>
<tr><td>Is outside of acceptable range (unacceptable)</td><td>Repeat quality control testing with the same control vial.</td></tr>
<tr><td>Is still unacceptable when repeated with the same control vial</td><td>Clean testing chamber and repeat testing with the same control vial.</td></tr>
<tr><td>Is still unacceptable after cleaning testing chamber and repeat testing with the same control vial</td><td>Repeat testing with a new control vial.
• If results are within acceptable range, proceed with patient testing by following the procedure block FRESH Mode Testing.
• If results are still unacceptable, notify a manager for further instructions.</td></tr>
</table>
</td></tr>
<tr><td>10</td><td>
• Select the problem associated with the test failure and press SAVE. It will then be recorded in the QC ARCHIVE with the corrective action noted.
• If the reason for the failure is not described on the list, note the reason and the corrective action taken in the USER DEFINED field.
• Click: REPORT to view and print the test results report.
</td></tr>
</table>

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

FRESH Mode Testing

Follow the steps below to perform and enter patient data on the SQA-VISION Analyzer for FRESH mode testing.

	Entering Patient Data
Step	**Action**
1	From the Home Screen, select TEST PATIENT.
2	Under the **FRESH** Tab, enter the PATIENT/SAMPLE DATA. Some information will be available in the *Semen Analysis – Patient Questionnaire Form* submitted with the sample.

PATIENT ID	Patient's medical record number.
PATIENT NAME and D.O.B	Patient's Full name and Date of Birth, previously run patients will auto-populate these fields when Patient ID is entered.
ABSTINENCE	Number of days since the patient's last ejaculation
SAMPLE ID/ ACCESSION #	Patient's sample accession number
COLLECTED Date and Time	Sample collection date and time
RECEIVED Date and Time	Sample received date and time
METHOD COLLECTION	Masturbation or special direction from physician
CONTAINER	Sterile Cup or other

Additional information can be entered *if applicable* by clicking ADDITIONAL button on the Test Patient window POST analysis such as:

COLLECTION ISSUES	Enter any collection issue(s).
TRANSPORT ISSUES	Enter any transport issue(s).
SEMEN APPEARANCE COMMENT	Enter any abnormal semen appearance observed.

Continued on next page

Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

FRESH Mode Testing, continued

	Entering Patient Data
Step	**Action**
3	**VOLUME** • Pour specimen into a graduated plastic centrifuge tube and determine the volume to the nearest 0.1 mL. • Enter the volume of the entire specimen (whole ejaculate) in milliliters.
4	**WBC CONC** Follow the package insert instructions for QwikCheck Test Strips to test for WBC. • <1 M/mL: Any color LIGHTER than the Leukocytes >= 1M/mL patch on the label is considered Leukocytes <1 M/mL which is considered normal. • >= 1M/mL: When the WBC concentration in semen is >= 1M/mL, the Leukocytes patch of the QwikCheck test strips reacts and reaches or exceeds the darkest color on the color chart which is considered abnormal.
5	**Ph** Use pH test strip to determine sample pH.
6	**APPEARANCE – NORM/ABNORM** Appearance is based on visual assessment of the specimen. • NORMAL – A normal liquefied semen sample has a homogeneous, grey-opalescent appearance. It may appear less opaque if the sperm concentration is very low; the color may also be different, i.e. yellow in a man with jaundice or taking certain vitamins or drugs. • ABNORMAL – The color of semen may be red-brown when red blood cells are present (haemospermia). Abnormal appearance may include significant quantities of debris, uric acid crystallization, opaque or thick seminal plasma and/or other significant abnormalities.

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

FRESH Mode Testing, continued

7	**LIQUEFACTION and VISCOSITY – NORM/ABNORM** WBC, pH and all other chemical tests should be completed before treating sample with QwikCheck Liquefaction kit. See package insert for instructions on how to use the kit. • NORM – Sample liquefies within 60 minutes at room temperature without using QwikCheck Liquefaction Kit. ABNORM – • If QwikCheck Liquefaction Kit successfully liquefied the sample within 60 minutes. • If QwikCheck Liquefaction Kit does not successfully liquefy the sample within 60 minutes.

Step	Entering Patient Data Action
	FRESH Mode Testing
1	After entering all the Patient and Sample Data, determine the volume of specimen to be tested and prepare for testing.

If the volume of specimen is…	Then…
Normal (≥0.5 mL)	Prepare a testing capillary for a normal volume specimen. Refer to procedure block ***Filling the SQA-V Testing Capillary.***

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

FRESH Mode Testing, continued

If the volume of specimen is…	Then…
Low volume with 0.3 mL - 0.5 mL	• A 1+1 dilution (1:2) is to be performed before testing the semen sample. • Use QwikCheck-Dilution Kit and dilute the semen 1:2. ***Note*:** Semen sample must be completely liquefied and well mixed prior to dilution. • Pipette **equal amount** (300 uL) of semen sample and QwikCheck Dilution in a wide mouth dilution container provided. • Gently rotate the container to evenly distribute the spermatozoa throughout the sample without introducing bubbles. To prevent air bubbles from forming, do not shake, or use a pipette to mix, or use a pipette to aspirate. • Fill a testing capillary in the usual manner for normal volume specimen. Refer to procedure block ***Filling the SQA-V Testing Capillary***. • Highlight the **1+1 DILUTION** button by clicking it.
<0.3 mL	Manual Counter can be performed using the visualization compartment. Testing volume is ~3.5 µL which is required for each fixed coverslip chamber. See procedure block ***Visualization Process – Low Quality and Manual Counters.***

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

FRESH Mode Testing, continued

	FRESH MODE Testing
Step	**Action**
2	Click **TEST NOW** to perform patient testing. ***Note:*** The system will Self Calibrate. Do not use the keypad or insert a testing capillary/slide at this time. 

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

FRESH Mode Testing, continued

	FRESH MODE Testing
Step	**Action**
3	• After system calibration, the instrument will instruct to insert testing capillary. Follow VISION instructions:  • A table of results will be displayed after testing Fresh semen samples with normal volume or diluted 1:2 samples. • Test Results are automatically saved. Printable test report can be opened by clicking the **Report** button. ***Notes:*** • Low quality test results that fall below the SQA-VISION dynamic range will automatically open the Low Quality Counter/ Visualization screen for manual assessment. • Only Sperm Concentration, Total Motile, Motile Sperm Concentration and SMI values will be reported automatically due to the limited number of sperm cells (i.e. Sperm Concentration <2 M/mL), very low motility and/or poor morphology. • To obtain more precise values and a full report, refer to procedure block ***Visualization Process – Low Quality and Manual Counters***.

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Cerner Resulting

After obtaining report from instrument, proceed to Accession Result Entry in Cerner for final verification of results. Follow steps below before clicking *Verify* of test results.

Step	Action	
	DTA	**Result**
1	Semen Collection Time	Result as obtained from the instrument
2	Days of Abstained	Result as obtained from the instrument
3	Method of Collection	Result as obtained from the instrument
4	Semen Collection Container	Result as obtained from the instrument
5	Collection Issues	Result as obtained from the instrument
6	Transport Issues	Result as obtained from the instrument
7	Specimen Received Time	Result as obtained from the instrument

8	Analysis Time	Result as obtained from the instrument
		• Skip Step 9 and proceed to Step 10 if sample is within 1 hour from collection.
		• Proceed to Step 9 if sample is:
		  • >1 hour from collection, or
		  • >2 hours from collection AND immotile

9	Analysis Time Comment	If sample is: • >1 hour but <2 hours from collection, then enter drop down selection result of: >1Hr from Collection; Motility results are questionable due to age of specimen. • >2 hours from collection AND immotile, then cancel using Cerner cancel message: Stability Exceeded, Test Not Performed. See Lab Informatics procedure for ***Canceling Test Orders*** in LabNet or MasterControl.

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Cerner Resulting, continued

	Step	Action
	DTA	**RESULT**
10	Semen Appearance	Result as obtained from the instrument
11	Semen Appearance Comment	Result as obtained from the instrument
12	Semen Liquefaction and Viscosity	Enter drop down selection result: • Normal if liquefied within 1 hour without addition of liquefaction kit. • Abn1 if liquefied within 1 hour with addition of liquefaction kit. • Abn>1 if not liquefied within 1 hour despite addition of liquefaction kit.
13	Semen pH	Result as obtained from the instrument
14	Semen WBC	Result as obtained from the instrument
15	Semen Volume	Result as obtained from the instrument
16	Sperm Concentration	Result as obtained from instrument. • proceed to step 17 if result is below instrument reportable range "<2 M/ml" • step 17 and proceed to step 18 if result is ≥ 2M/ml
17	Sperm Concentration Comment	After review of sample microscopically enter drop down selection result of: • **Rare Sperm/hpf** if sperm is seen. • **No Sperm/hpf** if no sperm is seen.
18	Immotility (IM)	Result as obtained from instrument.
19	Nonprogressive Motility (NP)	Result as obtained from instrument.
20	Progressive Motility (PR)	Result as obtained from instrument.
21	Tot PR Mot Cnt	Result as calculated by Cerner.
22	Norm Morph pct	Result as obtained from instrument.
23	Total Sperm/Ejaculation	Result as obtained from instrument.
24	Tot Motility (PR+NP)	Result as obtained from instrument.

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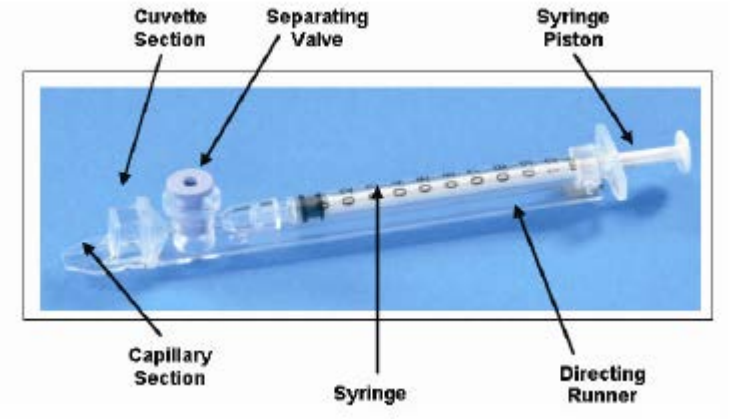
Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Filling the SQA-V Testing Capillary

Follow the steps below to fill the SQA-V testing capillary with a normal volume sample.



SQA-V Testing Capillary

Notes:

- Sample volume must be **at least** 0.5 mL.
- Sample container should be wide-necked and deep enough to facilitate inserting the capillary into the sample at the bottom of the container.
- Sample must be completely liquefied and well mixed prior to aspiration. Gently rotate container to fully mix liquefied sample.
  WARNING: Do not shake or use a pipette to aspirate and dispense sample in order to mix. Otherwise, air bubbles will form.
- Carefully check that liquefied, fully mixed sample is free of air bubbles (or that there is an adequate amount of sample below the air bubbles) before immersing the capillary into the sample, thus ensuring no air bubbles will be aspirated into the capillary.

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Filling the SQA-V Testing Capillary, continued

Step	Action
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. • 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. Continue to aspirate the sample until it appears in the Luer adaptor. 
2	• Holding the capillary in a vertical position, 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. 

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Filling the SQA-V Testing Capillary, continued

Step	Action
3	• Quickly (to avoid wicking) and thoroughly wipe the outer surface of the capillary, both top and bottom, with Kimwipe. • It is important to remove all semen from the exterior of the capillary to prevent the SQA-VISION 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 (a meniscus has formed in the thin part of the capillary), fill the capillary part from the cuvette section by slightly pushing in the piston.
4	• Slowly and carefully push-in the blue separating valve of the testing capillary until it is level with the plastic.  • The capillary is now ready to be inserted into the SQA-VISION measurement compartment for testing.
5	• Insert 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.

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Visualization System – Fixed Coverslip and Slide Preparations

The SQA-VISION Visualization System (Manual Method in SQA-VISION) is used to view and count sperm cells, capture static and dynamic images, and perform manual morphology assessment. The system displays real time videos or pictures of the semen sample on a PC monitor.

The visualization system:

- Accommodates a VISION fixed coverslip slide or a standard slide (both 20-micron depth).
- Allows smooth magnification transition from x1188 to x1725 (use Zoom In/Out).

	Fixed Coverslip Preparation
Step	**Action**
1	Mix the semen sample thoroughly and pipette **~3.5 µl** of semen. Pipetting the correct volume of sample required is critical for the accuracy of the count being performed.
2	Load the sample in the fixed coverslip as instructed by the arrows. ***Note:*** There are two wells on each slide for duplicate counts. 
3	After loading the sample, 'drop' the slide into the slide holder. 
4	Insert the slide holder into the VISION visualization compartment. 

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

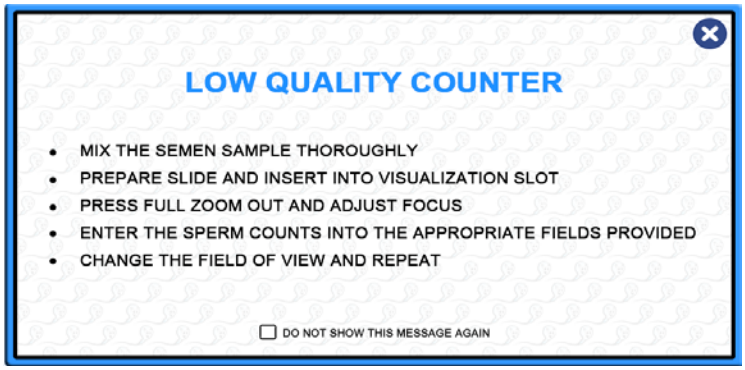
Visualization System – Fixed Coverslip and Slide Preparations, continued

	Standard Slide Preparation
Step	**Action**
1	Mix the semen sample thoroughly and load 10 µl of semen onto the **distal** end of a standard slide and cover with a 22 mm x 22 mm cover-slip (to insure 20 micron depth).
2	Insert the prepared standard slide into the SQA-VISION slide holder and insert into the visualization compartment of the VISION.

Visualization Process – Low Quality and Manual Counters

There are different counters that use the Visualization Process:

- **Low Quality Counter** – The visualization process is reflexed automatically during the testing process when Low Quality sample results fall below the dynamic range of SQA-VISION (i.e. Sperm Concentration <2M/mL). The manual counting results will be used to report final test results (requires a fixed coverslip slide).
- **Manual Counter** – Visualization process can be opened manually through Test Patient tab >> Manual Mode.

	Low Quality Counter
Step	**Action**
1	• Low Quality Counter instructions will automatically display when test results fall below the SQA-VISION dynamic range.  • Refer to procedure steps above for **Fixed Cover Slip Preparation** or **Standard Slide Preparation** to prepare slide and insert into visualization slot.

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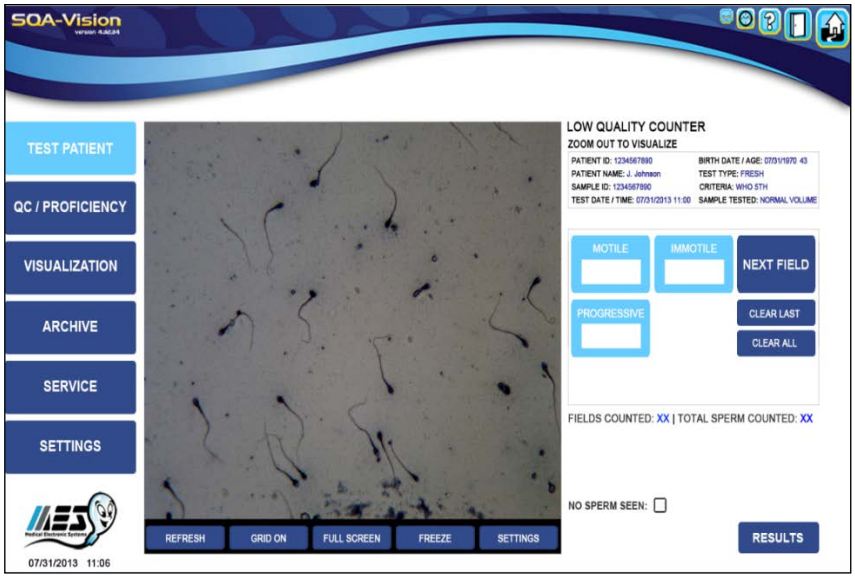
Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Visualization Process – Low Quality and Manual Counters, continued

	Low Quality Counter
Step	**Action**
2	Assess the number of **Motile(F9)**, **Immotile(F10)** and **Progressively Motile(F11)** spermatozoa in multiple fields of view and enter the number into the specified fields as displayed below. ***Notes:*** • It is important to fully **ZOOM OUT** before performing any count using visualization process. • The number of motile sperm must be greater than the number of Progressively Motile sperm. 
3	Click the **NEXT FIELD** button and turn the Visualization Field of View Stage knob to assess a new field of view.
4	Count a **minimum of 200 sperm cells** or **minimum of 20 fields** (whichever comes first) by turning the knob of the Field of View Stage to advance to the NEXT FIELD.
5	Activate the GRID ON, FULL SCREEN and FREEZE functions for easier counting. ***Note:*** During the process of counting, the Number of **FIELDS COUNTED** and **TOTAL SPERM COUNTED** will be shown on the screen.

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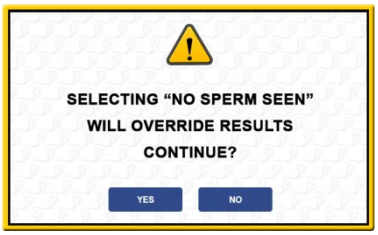
Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Visualization Process – Low Quality and Manual Counters, continued

	Low Quality Counter
Step	**Action**
6	• Note if sperm is seen. • Click NO SPERM SEEN if no spermatozoa were found in all fields of view. A warning message will be shown in this case.  .
7	Click the **RESULTS** button to finalize the manual assessment.
8	• Result should auto transmit to Cerner. • See procedure block ***Cerner Resulting*** for guide in verifying results.

Manual Counter:

- Manual Counter can be used as a back up method instead of using a microscope.
- Manual Counter will be used for low volume specimen below 0.3 mL.
- Manual Counter can also be used to review and compare automated results performed by the instrument.

	Manual Counter
Step	**Action**
1	Mix semen sample gently and thoroughly.
2	Prepare a 1:20 dilution of a liquefied semen sample. ***Example:*** 50uL semen sample + 950uL semen diluting fluid
3	Open **Manual Counter** by selecting **TEST PATIENT > MANUAL** from the main menu.

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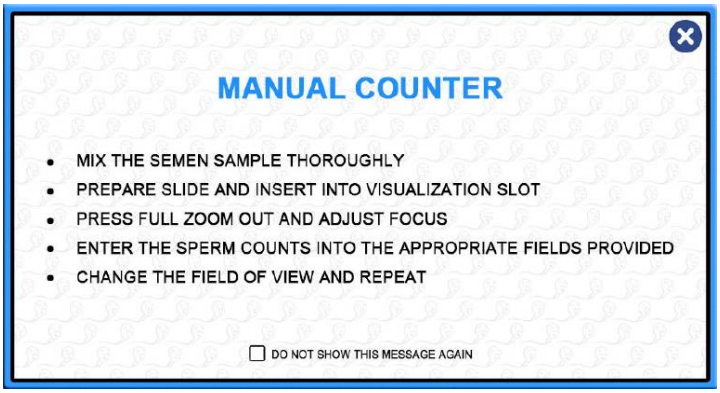
Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Visualization Process – Low Quality and Manual Counters, continued

	Manual Counter
Step	**Action**
4	After populating all the patient/sample information, click **TEST NOW** button and follow instructions on the screen. 
5	Under the Manual Counter window, select **Sample Dilution** performed.

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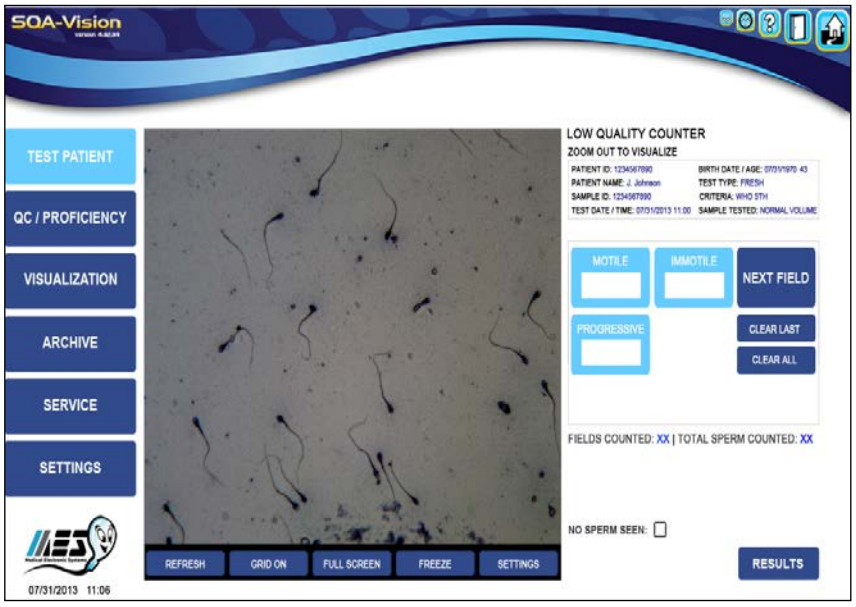
Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Visualization Process – Low Quality and Manual Counters, continued

	Manual Counter
Step	**Action**
6	Assess the number of **Motile(F9)**, **Immotile(F10)** and **Progressively Motile(F11)** spermatozoa in multiple fields of view and enter the number into the specified fields as displayed below. ***Notes:*** • It is important to fully zoom out before performing any count using visualization process. • The number of motile sperm must be greater than the number of Progressively Motile sperm. 
7	Click the **NEXT FIELD** button and turn the Visualization Field of View Stage knob to assess a new field of view.
8	Count a **minimum of 200 sperm cells** or **minimum of 20 fields** (whichever comes first) by turning the knob of the Field of View Stage to advance to the NEXT FIELD.

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Visualization Process – Low Quality and Manual Counters, continued

	Manual Counter
Step	**Action**
9	Activate the GRID ON, FULL SCREEN and FREEZE functions for easier counting. ***Note:*** During the process of counting, the Number of **FIELDS COUNTED** and **TOTAL SPERM COUNTED** will be shown on the screen.
10	• Note if sperm is seen. • Click NO SPERM SEEN if no spermatozoa were found in all fields of view. A warning message will be shown in this case.  .
11	Click the **RESULTS** button to finalize the manual assessment.
12	• Result should auto transmit to Cerner. • See procedure block ***Cerner Resulting*** for guide in verifying results.

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Reportable Range

The table below shows the reportable range for SQA-VISION automated results for FRESH sample type.

Sperm Concentration (M/mL)	<2 - 400
Motility (%)	0 - 100
Progressive PR (%)	≥ 32
Normal Forms Morph (%)	2 - 30
Sperm # (M/ejaculate)	≥ 39

Reference Range

- The ranges established by the SQA-VISION are based on WHO 5th reference values or MES (for proprietary semen parameters).
- The table below shows the reference ranges for Kaiser Permanente.

Semen Volume	>= 1.5 mL
Semen pH	>= 7.2
Semen WBC	<1 million/mL
Total Sperm/Ejaculation	>= 39 million
Sperm Concentration	>= 15 million/mL
Total Motility (PR+NP)	>= 40%
Progressive Motility (PR)	>= 32%
Morphology Normal Forms	>= 4%

Limitations

- Analysis should begin within 60 minutes of collection, otherwise the critical determination of motility and possible other parameters may not be reliable.
- Motility testing is time sensitive and is run FIRST on the SQA-VISION.
- Specimens received more than 60 minutes, but less than 2 hours after collection should be analyzed. Please note that results are questionable due to age of specimen.
- If specimen is not sufficient, report as QNS for those tests that were not completed.

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Regulatory Requirements to be Performed

The following regulatory requirements are to be performed:

- **Instrument Calibration Verification**: The analyzer's calibration is checked against the original factory calibration parameters for the following criteria:
    - At complete changes of reagents, unless it can be demonstrated that changing reagent lots does not affect either the range used to report patient test results or the control values
    - When QC materials reflect an unusual trend or shift or are outside acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem
    - After major maintenance or service
    - When recommended by the manufacturer
    - At least every six months
- **System Precision and Lower Limit Detection and Motility Method Verification** at least semi-annually:
    - The precision and lower limit detection ability of the SQA-VISION is confirmed by completing an abbreviated validation study.
    - It is also suggested that 5 samples be compared to the backup method for motility method verification.
- **Proficiency Testing**:
    - Laboratories are required to show proficiency across three main semen analysis parameters: Sperm concentration, motility and morphology. Available PT samples may not currently address motility or morphology due to natural limitations associated with shipping live samples.
    - Contact CAP for more information and ordering details.

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Automated Semen Analysis Using the SQA-VISION Semen Quality Analyzer, Continued

Non-Controlled Documents

The following non-controlled documents support this procedure.

- CAP Laboratory Accreditation Standards Checklist
- SQA-VISION User Guide, Version 104.13.2, Catalog # VS-ML-01051-00 SQA VISION, February 28, 2016
- Product Insert; Medical Electronic Systems, QwikCheck Beads
- Product Insert; Medical Electronic Systems, QwikCheck Test Strips
- Product Insert; Medical Electronic Systems, QwikCheck Liquefaction
- Product Insert; Medical Electronic Systems, QwikCheck Dilution
- Technical Release Bulletin: Semi-Annual (every 6 months) Calibration Confirmation; Application: Any SQA-V/SPERMALITE Visualization System; Re-Issue date/Distribution: Tuesday, October 22nd, 2013/All SQA-V Users
- WHO laboratory manual for the Examination and processing of human semen, 5th Edition

Controlled Documents

The following controlled documents support this procedure.

Procedure
Semen Analysis Collection from local laboratory or LabNet
Procedure for Managing the Semen Analysis – Patient Questionnaire Form
Form
Daily Maintenance and Inspection (Model: SPERMALITE SQA-VISION)
Semen Analysis – Patient Questionnaire Form