SUBJECT: **SQA-V Gold Semen Analyzer**
7500.CC.H67

DEPARTMENT(S): Hematology

DATE APPROVED:

APPROVERS: Kevin Ferguson, MD

Applies to:		
☒ Santa Maria Campus, Marian Regional Medical Center	☐ Arroyo Grande Campus, Marian Regional Medical Center	☒ French Hospital Medical Center
☐ St. John's Pleasant Valley Hospital	☐ St. John's Regional Medical Center	

I. Purpose:

A semen analysis is performed in order to determine the fertility potential of a male.

II. clinical Complexity:

Moderate Complexity

III. Principle:

SQA-V measures light absorption by sperm cells to determine sperm concentration. Electrical signals generated by motile sperm cells moving through a light path create interruptions in the beam which are used to determine motility and the analysis between motility, functional sperm concentration and progressive motility are used to calculate percent normal morphology. Proprietary algorithms translate the collected data into test results.

IV. Specimen Requirements:

A. Specimens are collected in sterile containers. Confirm the patient's first and last name and date of birth are on the label or container.

B. Semen samples should be collected by masturbation after an abstinence period of no less than 2 days and no more than 7 days. Lubricant or condoms must not be used, vaginal or oral secretions must be avoided. The entire ejaculate must be collected.

C. Specimens should be kept at body temperature and delivered to the laboratory preferably within 30 minutes but will be accepted up to 1 hour post collection.

D. Unlabelled specimens, specimens received >2 hours post-collection, specimens that do not include the entire ejaculate and specimens in unapproved containers will not be accepted.

E. See Semen Analysis Patient Instructions form for additional information on proper semen collection.

F. Any problems associated with the semen collection or transport process identified by the patient are documented on the report as a result comment.

V. Materials:

Reagents / Media	Supplies / Materials	Equipment
● QwikCheck™ Liquefaction kit (store @ 22-26°C) ● QwikCheck™ Dilution Kits (store unopened bottle @ 22-26°C. Store at 4°C after opening) ● QwikCheck™ Beads (store @ 15-30°C) ● Qwikcheck™ Test Strip QC (Store @ 15-30°C)	● QwikCheck™ Test Strips (store @ 15-30°C) ● SQA-V cleaning kit ● Vortex mixer ● Plastic Pipette ● Kimwipes	● SQA-V capillaries ● SQA-V Gold Semen Analysis Analyzer ● Heat Block or Incubator (37°C)

VI. Calibration:

A. The SQA-V automatically performs a five minute Auto-Calibration and Self-Test when the system is turned on from both the back panel and the keypad.

Note: When the MAIN MENU appears, the SQA-V is ready for testing

1. Turn on the V-Sperm computer at this time and log into V-Sperm.
2. If the SQA-V GOLD requires additional tests to be loaded, please see the User Guide for instructions

VII. Maintenance:

A. Frequency:

1. Days when testing is performed
2. After every 10-15 tests and/or ANY spillage
3. If the system fails SELF-TEST
4. If the SQA-V becomes contaminated with semen

B. Daily:

1. Insert the long cleaning brush (bristle side down) all the way into the upper portion of the sample testing compartment.
2. Pull the brush out, applying downward pressure to sweep off the optics - a shelf will be felt in the back/top of the chamber. Repeat twice more.
3. Monitor the systems ‘REF. 2’ parameter. It should be between 2,800 and 3,200 mV for optimal performance.

C. Weekly:

1. Select the fibrous cleaning paddle from the SQA-V Cleaning Kit.
2. Moisten the fibrous side with ONE drop of cleaning fluid
3. Shake off excess fluid
4. Insert into the testing chamber (measurement compartment) with the fibrous material facing DOWN
5. Move the cleaning paddle in and out three times
6. Insert the sponge-tipped drying paddle into the testing chamber and leave for 10 seconds

   Note: Do not move the drying paddle in and out

VIII. Quality Control:

A. QwikCheck™ Test Strip Quality Control:

Use QwikChek Test Strip QC material to validate the QwikCheck Test Strips each day of patient testing. Once opened, reagent strips are stable for 90 days and controls are stable for 45 days. Write the open date and open expiration dates on the bottles.

1. There are 2 levels of QC indicated by a blue cap and a green cap.
2. Deliver 1 drop of each external quality control material onto the pH and WBC test pads on two different strips. Wait 1 minute to read the pH and 2 minutes to read the WBC results before comparing the pads to the color chart on the QwikCheck test strip label.
3. Confirm pH values and WBC concentration are within range listed on insert of control material.
4. Perform a visual quality control check of test strips. The test patches should appear white (leukocyte) and yellow (pH).
    - Green cap control: pH 5-6 and WBC negative
    - Blue cap control: pH 7-8 and WBC positive
5. Record date, lot numbers, expiration, and results on QwikCheck Test Strip Log with patient label.

B. QwikCheck™ Beads Control Material:

Run QwikCheck™ beads once per day of testing. Once opened, controls are stable for 90 days. Mark the open and expiration date on the control vials.

1. With each new lot number or shipment of QwikCheck-beads, follow the SQA-V User Guide instructions (or see Appendix 1 at the end of this SOP) for setting up the testing defaults based on the product labeling.
2. Select: RUN CONTROLS from the MAIN MENU of the SQA-V.
3. Select CONTROL LEVEL: #1, #2 or NEGATIVE CONTROL based on the sample to be run.
4. Press ENTER to continue
5. Before opening, thoroughly mix or vortex the QwikCheck-beads. It is imperative that the beads are evenly mixed, without creating bubbles, in order to ensure accurate results.
6. The negative control does not require extensive mixing.
7. Following the SQA-V on-screen instructions for "Controls"
8. Open the beads and immediately aspirate the beads into the 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.
9. Following the SQA-V on-screen instructions for "Controls" insert the SQA-V capillary into the SQA-V in the same manner you would test a normal sample of semen, being sure to wipe free of any sample before insertion.
10. Immediately close the container tightly after use to avoid evaporation or spillage.
11. Testing will begin automatically.
12. Print and import Control test results.

C. Running Low level Stabilized Sperm Samples

1. If a stabilized sperm sample run on the SQA-V Gold in the stabilized sperm QUALITY CONTROL MODE results in zero (beyond 2M/ml dynamic range) Rerun the sample in the fresh mode.
2. Go to MAIN MENU > TEST NEW PATIENT.
3. Enter the Proficiency Sample Number as the patient identification.
4. Enter the date of testing as the date of birth.
5. On the next screen select FRESH.
6. Select WBC<1M/ml.
7. Select YES when asked “IS SAMPLE VOLUME SUFFICIENT FOR COMPLETE TESTING ≥.5 ml?”
8. VORTEX the stabilized sperm sample.
9. Transfer the sample from the original vial to the 10 ml collection cup and mark the cup with the proficiency testing identification number.
10. Mix the sample and immediately fill the SQA-V capillary and run the test.

IX. Procedure:

A. Patient Specimen Procedure:

1. From the MAIN MENU select: TEST NEW PATIENT and press ENTER
2. ENTER PATIENT/SAMPLE DATA in the screen below.
3. Patient ID - unique number identifying the patient
4. Birth Date - Birth date of the patient
5. Abstinence - # of days since the patient's last ejaculation
6. Sample/Accession # - enter the LIS barcode number
7. Collected - the date and time the sample was collected
8. Received - the date and time the sample was received.
9. Press ENTER to view the SAMPLE TYPE screen:
10. Select: Sample Type (required entry) based on the following options only:
    a) Fresh Samples - tested within an hour of collection and not enriched, diluted or treated.
    b) Postvasectomy - Fresh samples tested within one hour of collection and designated as postvasectomy specimens. (Please note: A separate Postvasectomy SOP has been defined for this type of Sample Testing – Please see specific SOP).
    c) WASHED and FROZEN – SAMPLES NOT PERFORMED
11. Enter the following information:
    a) VOLUME - REQUIRED ENTRY The volume of the whole ejaculate in milliliters
        1) Measure the volume of the sample to the nearest 0.5 mL by transferring the entire specimen into a graduated conical tube.
        2) Transfer the entire specimen back to the original container.
    b) WBC CONC – REQUIRED ENTRY Select < = 1M/ml (normal) or > 1M/ml (abnormal) leukocytes (see how to test for this below)

c) QwikCheck™ Test Strips for Semen Analysis Leukocytes and pH

1) Place one drop of semen on each of the two test patches
2) Wait 1 minute for pH and 2 minutes for leukocyte (WBC concentration)
3) Compare leukocyte and pH results to the color chart provided on the product label
4) WBC's: If < 1 M/ml (white) select Normal or if >= 1M/ml (dark lavender) select Abnormal (Abnorm) in the Sample Data screen
5) pH: Enter the number most closely associated with the color of the patch

d) LIQUIFICATION/VISCOSITY – NORM/ABNORM (Norm - liquefies within 60 minutes @ room temperature)

1) After liquefaction, the viscosity of the sample is estimated by gently aspirating it into a plastic disposable pipette and observing the semen drop off the pipette tip back into the container.
   a. A normal sample leaves the pipette in small discrete drops.
   b. If viscosity is abnormal, the drop will form a thread more than 2 cm long
2) QwikCheck™Liquefaction can be used to accelerate the liquefaction of viscous samples:
   a. Select one vial of QwikCheck liquefaction powder
   b. Tap the vial to move the contents to the bottom of the vial before opening
   c. Add the entire contents of one vial to a viscous semen sample
   d. Gently mix to dissolve the powder
   e. Once the sample has liquefied (5-10 minutes) test in the SQA-V

12. Using the left/right arrow keys on the keypad of the SQA-V select:

a) YES, for NORMAL VOLUME samples ≥0.5mL

b) ENTER to select

IS SAMPLE VOLUME SUFFICIENT FOR COMPLETE TESTING ≥ 0.5mL?
YES/NO

c) NORMAL VOLUME SPECIMENS – After selecting YES and ENTER, the screen below will be displayed:

Fresh
Normal Volume Specimen
Fill Clean and Wipe Capillary
AUTOCALIBRATION – DO NOT TOUCH UNIT

1) Prepare a testing capillary for a normal volume sample by inserting the Capillary into the specimen cup.
2) When the AUTOCALIBRATION is finished (about 15 seconds) and the screen will say "INSERT INTO TESTING CHAMBER":

a. Testing should be performed on a room temperature sample. Before inserting capillary into the testing chamber, it is recommended to visualize the sample in the UPPER visualization chamber to confirm sample composition. If few or no sperm are seen, press the ESCAPE key to back out from the testing screen and repeat in “POSTVASECTOMY” mode – this will ensure the greatest possible accuracy in sample analysis. For low quality test results repeat in ‘POSTVASECTOMY” mode.

b. Samples should also be observed for significant quantities of debris, uric acid crystallization, opaque or thick seminal plasma and/or other abnormalities. Such cases should be noted on the report and/or confirmed by manual observation.

3) Wipe the capillary free of sample and ensure the chamber is free from bubbles before insertion. Insert the testing capillary with BLUE STOPPER pointing down in the LOWER measurement compartment of the SQA-V.

4) Testing will begin automatically when the capillary is inserted and the screen will display a time bar during the testing cycle. Do not touch the system during the testing cycle.

NORMAL VOLUME SPECIMEN FILL, CLEAN AND WIPE CAPILLARY

d) LOW VOLUME SPECIMENS - After selecting NO and ENTER, the screen below will be displayed:

LOW VOLUME SPECIMEN PLEASE SELECT SAMPLE TESTING OPTION: DILUTE SEMEN 1:1 WITH MEDIA LOW VOLUME – 20 MICROLITERS

1) Select the option: DILUTE SEMEN 1:1 WITH MEDIA

2) ENTER and use QwikCheck-Dilution Kit and dilute the semen 1:1 following the directions displayed on the next screen.

LOW VOLUME SPECIMEN 1. DILUTE SEMEN 1:1 WITH MEDIA 2. MIX SAMPLE THOROUGHLY 3. FILL, CLEAN AND WIPE CAPILLARY

3) Measure the volume of the neat semen sample.

4) Dilute 1:1 with QwikCheck-Dilution

5) Thoroughly mix the sample in order to evenly distribute the spermatozoa throughout the sample without introducing bubbles (do not use a pipette to mix)

6) Fill a testing capillary in the usual manner (Normal Volume) and insert it into the testing compartment of the SQA-V with the blue stopper facing down.

B. Testing Patient Samples:

1. Once the capillary is inserted into the SQA-V testing compartment (with the blue stopper facing downwards), testing will begin automatically.
2. The testing cycle takes about 75 seconds.
3. If the sample is of low quality an extra 2 minute testing cycle will begin automatically (A low quality specimen is defined as having a concentration of < 5 M/ml sperm or no motile cells).
4. DO NOT TOUCH THE SYSTEM during the testing cycle.
5. Insert the testing capillary in the UPPER visual compartment of the SQA-V to CONFIRM Test Results (correlate motility and concentration to visual observation).
6. The two screens below will display complete test results:

TEST RESULTS SPERM CONC. 32.6 M/mL MOTILITY <a+b+c> 28.0% RAPID PROG. MOTILITY <a> 5.2% SLOW PROG. MOTILITY <b> 14.1%
TEST RESULTS MSC 9.1 M/Ml FSC 2.5 M/mL PMSC <a> 1.7 M/mL VELOCITY 9 mic/sec PMSC <b> 4.6 M/mL SMI 34 TOTALS PER EJACULATE

7. Test results will be automatically saved and printed by the SQA-V.
8. The screen below will be displayed. Press “IMPORT TEST” on the SQA-V computer screen.

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

X. Result Reporting:

A. Reference Range

Table 1. Reference range

Parameter	>= 5th Percentile	Normal Reference Range
Semen volume (ml)	1.4	1.3-1.5
Sperm Concentration (M/ml)	16	15-18

Total sperm number (M)	39	35-40
Total motility (%)	42	40-43
Progressive motility (%)	30	29-31
Morphology normal forms (%)	4	3.9-4.0
pH	7.2	>=7.2
WBC Concentration		<1

WHO 2021 (6th Ed) lower fifth percentile (from men in reference population) and normal ranges established using 95% confidence interval.

B. Reporting in SQA:

1. Enter the patient's first and last name and confirm the correct birthday.
2. Enter ordering Doctor and person performing and person releasing test.
3. Attach Video/Picture to test record (click PREVIEW VIDEO button->click CAPTURE PICTURE or VIDEO->click STOP CAPTURING->click OK to end).
4. Print report and save the test results.
5. Enter results into the computer system.
6. Release results from the computer system.
7. Print released results using the computer system if desired.

C. Reporting in Cerner:

1. Enter time received, time of collection, semen volume, pH and total sperm in Cerner
2. Use the drop down menu to enter “See scanned report” under liquification, motility, and %normal sperm.
3. Select all results and under batch comments select ‘Result Comment’ and include the comment “See scanned report for complete semen analysis results, including reference ranges”.
4. Scan SQA printout under results attached and fax results if the provider is not able to access Cerner. For low quality samples (<2 M/ml) replace the second page of SQA printout with the second page of ‘POSTVASECTOMY’ printout-line out Postvasectomy.

XI. Limitation of Procedure:

A. Analysis should begin within 60 minutes of collection, otherwise the critical determination of motility and possibly other parameters may not be reliable.

B. Motility testing is time sensitive. Specimens received more than one hour, but less than two hours after collection should be analyzed.

C. If the semen sample is not sufficient for even LOW VOLUME testing (<250 µl), append the abbreviation QNS (under result comment include “Quantity not sufficient to perform testing”) to those tests that were not completed.

D. In rare circumstances, fresh samples can be tested using ‘Washed’ in place of ‘Fresh’ mode; this is useful for watery samples that may have a spuriously low sperm concentration and high motility per MES service specialist.

E. Stabilized Sperm Proficiency Challenges: SQA Technical Bulletin (April 27, 2014) reports fixative material used in the preservation of proficiency testing samples has been known to change the matrix and size of spermatozoa in the proficiency sample.

1. Spermatozoa are shrunk because of fixation which causes a change to their size and shape.
2. The seminal plasma is diluted with a fixative or completely replaced with a media causing a decrease in the sample viscosity. The SQA-V algorithm works based on the optical density of SEMINAL PLASMA (not the dilution media or modified ‘seminal plasma' used in stabilized sperm).
3. A decrease in sample viscosity and density results in rapid cell sedimentation and uneven distribution of the cells throughout the volume of sample. These factors may result in inconsistent concentration of cells in the samples received by the labs and in aliquots taken for analysis. The sample tested may no longer reflect the target value estimated by the manufacturer.
4. All of these facts will adversely impact the accuracy of automated testing especially in low concentration samples because the prepared quality control material is not the same as a normal semen sample. The SQA-V is designed to run semen samples within one hour of collection and the concentration dynamic range of the SQA-V is 2M/ml on the low end. Therefore, running a quality control sample of very low quality on the SQA-V, may result in a zero reading.

F. The lower fifth percentile should not be interpreted as a true divide between fertility and infertility. There is a large overlap between multiparametric interpretation of results between fertile and infertile men.

XII. References

A. Medial Electronic Systems LLC; SQA-V GOLD User Guide

B. Package insert; Medical Electronic Systems, Qwik-Check Beads

C. Package insert; Medical Electronic Systems, Qwik-Check Test Strips

D. Package insert; Medical Electronic Systems, Qwik-Check Liquefaction Kits

E. Package insert; Medical Electronic Systems, Qwik-Check Dilution

F. SQA Gold Semen Analysis SOP. January 2015

G. Technical Release Bulletin: Stabilized Sperm Proficiency Challenge for LOW LEVEL Target Values. 4/27/14

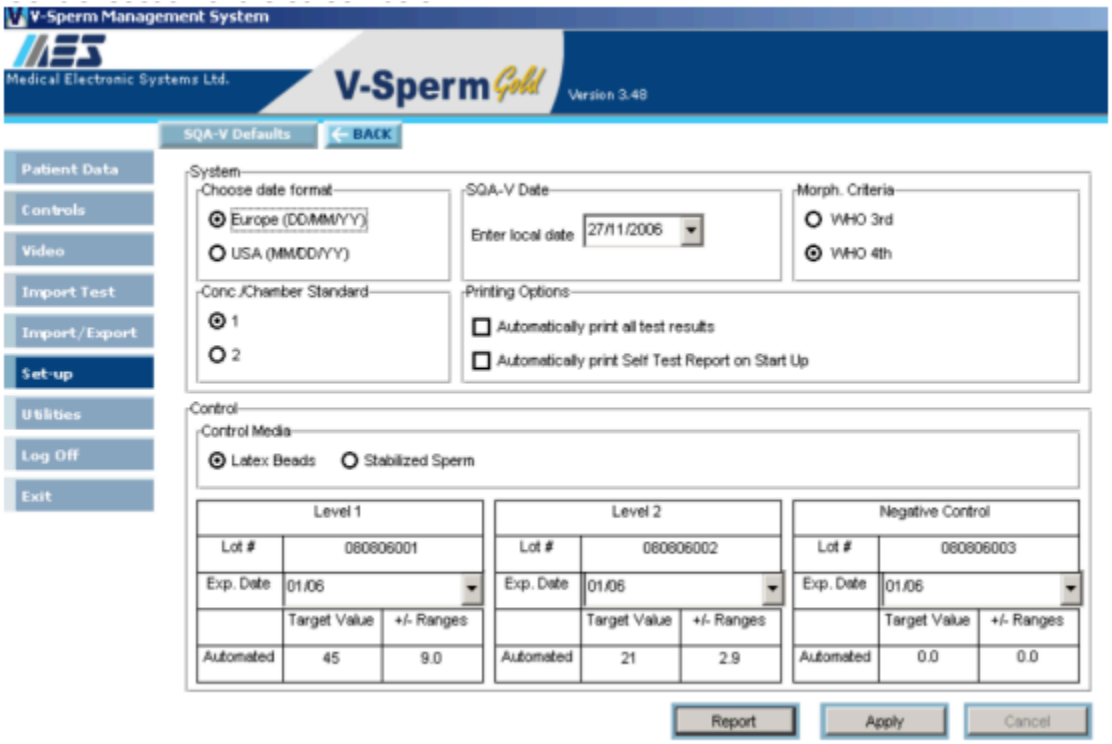
H. WHO laboratory manual for the examination and processing of human semen-6th ed., WHO Press, 2021.

I. WHO laboratory manual for the examination and processing of human semen-5th ed., WHO Press, 2010.

XIII. Appendixes

Appendix 1. Setting up a NEW BOX of Qwik-Check Beads Controls

A. Each time a new lot of Qwik-Check Beads (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.
    a. From the SQA-V MAIN MENU select SERVICE>SERVICE DATA
    b. Activate the V-Sperm GOLD on the PC and select: SET-UP>SQA-V>SQA-V Defaults and press CONTINUE
    c. The set-up screen below will be activated in V-Sperm GOLD on the PC. Go to the Control section of the screen below



    d. Select: Latex Beads
    e. Enter the following information from the box labeling:
        i. **LOT#** - number identifying the control media lot.
        ii. **EXP. DATE** - control expiration date (MM=month, YY=year)
        iii. **TARGET VALUE** and +/- RANGE - manufacturer's "Target Value and +/- Range" for the SQA-V Automated System
        iv. **NEGATIVE** control target values and +/- ranges are pre-set to 0.0
    f. Click on APPLY - The set-up may take two minutes.

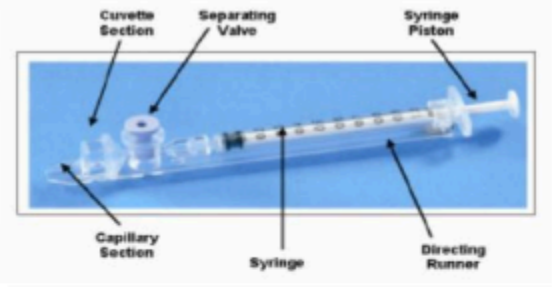
Appendix 2. Loading the SQA-V Testing Capillary

SQA-V Gold Filling the SQA-V Capillary with a Normal or Low Volume Sample

Filling the SQA-V Capillary with a Normal Volume Sample

Before filling the capillary please note:

- Sample volume should be **at least .5 ml** If not, see instructions for filling the capillary with a low volume sample.
- Mix the sample in the collection container by gentle rotation in order not to introduce any air bubbles.
- The semen sample must be **completely liquefied.**



Filling the capillary:

1. **Push the syringe piston in fully**. Place only thin part of the capillary into the bottom of the sample at an angle of about 45 degrees (Figure 1).
2. Place two fingers below the syringe piston and **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.
3. **Visually confirm that the sample has completely filled** the testing capillary and is without a meniscus in the thin capillary section (Figure 2). **Tap on the syringe to make sure there are no air bubbles** in the sample. If, after tapping, air bubbles appear in the thin or the cuvette section, aspirate additional semen to draw the air bubbles into the syringe.
4. Quickly and **thoroughly wipe the outer surface of the capillary** (Figure 3). Remove all semen from the exterior of the capillary to prevent spillage into the SQA-V optical chamber. If, after wiping a meniscus has formed in the thin part of the capillary, back-fill the capillary by slightly pushing in the piston.

Figure 3

5. **Push-in the blue separating valve** of the testing capillary until it is level with the plastic (Figure 4).
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.**

Figure 1

Figure 2: Fill the capillary until the sample appears above the Leur adaptor. Inspect for air bubbles in the capillary and cuvette sections.

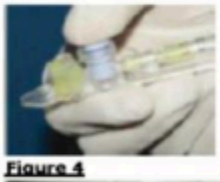
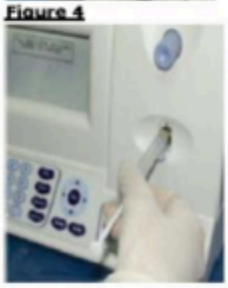
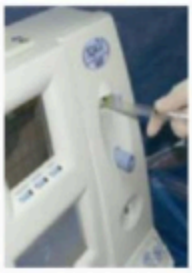


Figure 4





Appendix 3. Using the SQA-V Visualization System:

A. To view the SQA-V testing capillary in the visualization system:
    a. Fill the SQA-V testing capillary and insert the testing capillary with the BLUE STOPPER facing upwards (opposite of insertion during testing)
B. Visualization Process:
    a. The video display will automatically illuminate when the SQA-V is turned on.
    b. Wait for the self-test to complete (system is disabled at this time).
    c. To ensure that the visualization system is working properly prior to use:
        i. Press the HIGH ILLUMINATION key multiple times to ensure a maximum level setting.
        ii. Turn BRIGHTNESS, CONTRAST, and COLOR buttons all the way counterclockwise.
        iii. Turn the FOCUS knob fully clockwise.
        iv. To view cells: Press ZOOM IN to maximum magnification (x500).
        v. To count cells: Press ZOOM OUT to minimum magnification (x300).
    d. Insert semen sample capillary into the visualization chamber.
    e. Turn BRIGHTNESS knob clockwise until the video screen begins to lighten-up.
    f. Turn the FOCUS knob counterclockwise until the image is focused.
    g. Adjust CONTRAST, COLOR, BRIGHTNESS, FOCUS, and object ILLUMINATION controls for optimal image quality.
    h. Use ZOOM OUT (x300) / ZOOM IN (x500) to regulate magnification.
C. Counting Cells Using the Visualization Screen:
    a. Scan ten fields of the SQA-V capillary
    b. Enter the number of motile and immotile sperm cells visualized
    c. The final test results will report the greater number of cells found in the automated or visualization test.
    d. Leave the testing capillary in the visualization system
    e. Save the test to the SQA-V achieve and import it to the V-Sperm GOLD software.
    f. Following the V-Sperm user guide instructions, import the test into the V-Sperm database 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 a POSTVASECTOMY > 30 motile spermatozoa is equivalent to MSC > 2M/ml.