Procedure
Dignity Health Central Coast Service Area

**SUBJECT**: Post Vasectomy Semen Analysis

**ORIGIN**: Hematology

**NUMBER**: 7500.CC.H.68

|  |
| --- |
| **Applies to:** |
| [x]  Santa Maria Campus,Marian Regional Medical Center | [ ] Arroyo Grande Campus,Marian Regional Medical Center | [x] French Hospital Medical Center |
| [ ] St. John’s Pleasant Valley Hospital | [ ] St. John’s Regional Medical Center |

# PURPOSE:

To provide a procedure for the examination of post vasectomy semen samples for the detection of presence or absence of sperm.

# clinical complexity:

High Complexity

# Principle:

Following a vasectomy, the sperm count in semen ideally should be zero (azoospermia) within 12 weeks after the procedure. Although low numbers of immotile sperm can persist for months in some men, the presence of even a single motile sperm is evidence of an unsuccessful vasectomy. Post vasectomy semen samples are run on the SQA-V Analyzer with a five minute post vasectomy test that can detect the presence of a very small number of motile cells. Once the automated test has been performed the sample is visually scanned on the analyzer and by manual microscopy for the presence of motile and immotile sperm.

# EQUIPMENT and materials:

## SQA-V Gold Semen Analysis Analyzer

### Microscope

### Heat Block

### SQA-V capillaries

### QwikCheck™ Test Strips (store @ 15-30°C)

### QwikCheck™ Liquefaction kit (store @ 22-26°C)

### QwikCheck™ Dilution Kits (store unopened bottle @ 22-26°C. Store at 4°C after opening)

### QwikCheck™™ QC Beads (store @ 15-30°C) and WBC/pH Test Strips QC

### SQA-V cleaning kit

### Plastic Pipette

### 10 ul pipette and tips

### Glass microscope slides and 22mm x 22mm glass coverslip

# Calibration:

## The SQA-V automatically performs a five minute Auto-Calibration and Self-Test when the system is turned on from either the switch located in the rear of the instrument or the on/off button in the front of the instrument.

## Perform and record all instrument maintenance and function checks in accordance with each corresponding operating procedure.

## Record all auto-calibration, self-tests, and maintenance on the appropriate maintenance log.

## Microscope stages are cleaned as needed with alcohol pads to remove oil, dirt, and debris. Microscope objectives and oculars are cleaned as needed with lens cleaner and lens paper to remove oil, dirt, and debris.

## Preventive maintenance is performed and documented annually on each laboratory microscope to maintain optical alignment and proper functionality.

# Specimen Collection:

## Physician instructions should be followed regarding the length of time and number of ejaculations after the vasectomy procedure before a specimen for post vasectomy analysis is submitted.

## Specimens are collected in a sterile container supplied by the laboratory or physician office.

## Semen samples should be collected by masturbation after an abstinence period of no less than 48 hours and no more than seven days. Specimens should be maintained at body temperature before delivery to the laboratory.

## Specimens are to be delivered to the laboratory preferably within 30 minutes but will be accepted up to 1 hour post collection.

## Unlabeled specimens, specimens received >2 hours post-collection, and specimens in unapproved containers will not be accepted.

## See attached Semen Analysis Patient instruction form for additional information on proper semen collection. Patient instruction form is to be retained.

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

# Quality Control:

**QwikCheck™ Test Strip External Quality Control:**

Use external quality control material to validate the QwikCheck™ Test Strips each day of patient testing. Once opened, Reagent Strips are stable for 90 days. Mark the open and expiration date on the control vials.

### If using QwikCheck™ Test Strips QC then reconstitute one set of POS and NEG controls according to product insert.

### Use two pipettes to deliver one drop of mixed Negative QwikCheck™ Test Strips QC (or IRISpec CA) and another to deliver one drop of mixed Positive QwikCheck™ Test Strips QC (or IRISpec CB) to pH test pads on two different strips. Wait 60 seconds before comparing pad to color chart.

### Confirm pH and WBC values are within range listed on insert of control material.

### Perform a visual quality control check of test strips. The test patches should appear white (leukocyte) and yellow (pH).

### Record date, lot numbers, expiration, and results on QwikCheck™ Test Strip Log.

### **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.

### Select: RUN CONTROLS from the MAIN MENU of the SQA-V.

### Select CONTROL LEVEL: #1, #2 or NEGATIVE CONTROL based on the sample to be run

1. Press ENTER to continue
2. Before opening, thoroughly mix or vortex the QwikCheck™-beads. It is imperative that the beads are evenly mixed, without creating bubbles, in order to insure accurate results.
3. The negative control does not require extensive mixing.

### Following the SQA-V on-screen instructions for "Controls"

### 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.

### 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.

### Immediately close the container tightly after use to avoid evaporation or spillage.

### Testing will begin automatically.

### Print and import Control test results.

## It is each operator’s responsibility to ensure that quality control has been performed in accordance with procedure and is within acceptable range before running patient samples. Do not report patient results if quality control is not within an acceptable range.

**Running Low level Stabilized Sperm Samples**

### 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.

### Go to MAIN MENU > TEST NEW PATIENT.

### Enter the Proficiency Sample Number as the patient identification.

### Enter the date of testing as the date of birth.

### On the next screen select FRESH.

### Select WBC<1M/ml.

### Select YES when asked “IS SAMPLE VOLUME SUFFICIENT FOR COMPLETE TESTING ≥0.5 ml?”

### **VORTEX** the stabilized sperm sample.

### Transfer the sample from the original vial to the 10 ml collection cup and mark the cup with the proficiency testing identification number.

### Mix the sample and immediately fill the SQA-V capillary and run the test.

# Viii. Procedure:

## Semen samples are immediately delivered to the laboratory and incubated at 37°C until ready for testing.

## Semen analysis testing is performed as soon as the sample liquefies. Most semen samples should spontaneously liquefy within 30 minutes of collection but may take up to 60 minutes.

## The liquefaction is considered abnormal if the sample does not liquefy within 60 minutes of collection. Follow the QuikCheck Liquefaction procedure listed in 7500.CC.H.67 for samples that do not liquefy within 60 minutes.

## 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 normal sample leaves the pipette in small discrete drops. If viscosity is abnormal, the drop will form a thread more than 2 cm long.

## Measure the volume of the sample to the nearest 0.5 mL by transferring entire specimen into a graduated conical tube. Transfer entire specimen back to original container.

## Thoroughly mix the sample by aspirating the sample 10 times into a disposable plastic pipette. Do not introduce air bubbles. Do not mix with a vortex.

## Follow the QuikCheck™ Test Strip procedure listed in 7500.CC.H.67 to measure the level of leukocytes and determine the pH.

## **SQA-V Analysis:**

## Follow the Patient Specimen Procedure listed in 7500.CC.H.67 to begin testing with the SQA Analyzer. When prompted, enter all patient identifiers as well as all the sample information from the initial macroscopic evaluation.

## When prompted for Sample Type, select POSTVASECTOMY.

## If the specimen has a volume of at least 0.5 ml, ensure the sample is well mixed and prepare a testing capillary according to procedure. If the sample volume is <0.5 ml, follow the Low Volume Specimen procedure listed in 7500.CC.H.67 to dilute the sample.

## **NOTE:** Allow the sample to cool to room temperature (22-26 °C) before SQA-V testing.

## When prompted, insert the testing capillary into the LOWER measurement compartment of the SQA-V, making sure the BLUE STOPPER of the capillary is pointing down and the capillary has been completely wiped free of sample before insertion. Ensure no bubbles are present in the testing capillary.

## 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.

## The SQA-V runs a five minute POSTVASECTOMY test that can detect the presence of a very small number of motile cells. If present, test results for motile sperm are reported.

## After the testing is complete, the analyzer displays “ENTER VISUAL DATA PER USER GUIDE?” Select YES and press ENTER.

## Move the testing capillary from the bottom compartment and insert it into the upper visualization compartment with the BLUE STOPPER pointing up.

## Set the magnification to x300 by pressing the ZOOM OUT button until it stops. Press ENTER to continue.

## Turn the FOCUS knob fully counterclockwise. Begin to turn the focus knob in the opposite direction so the image comes back into focus. Slowly turn the focus knob in the same direction and scan the image for motile and immotile sperm. When the on-screen image becomes unfocused again, the scan procedure is complete. This procedure allows the user to scan the entire depth of the capillary (a total of 10 fields) for motile and immotile sperm.

## At the appropriate prompt, enter the number of Motile Sperm and Immotile Sperm visualized in all 10 fields.

## The SQA-V will automatically report the GREATER Number of cells found by the Automated or Visualization system.

## **NOTE:** 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 sperm is equivalent to MSC > 2 M/ml. Perform a normal (FRESH) semen analysis according to procedure.

## Press Enter on the SQA-V analyzer. The test results will print and the test result screen is displayed.

## **V-Sperm:**

## At the prompt, transfer the test results to the V-Sperm software by clicking IMPORT TEST button from the V-Sperm main menu.

## Once imported, the Patient Data Screen will display. Verify that the patient information is correct by comparing the on-screen patient information with the information on the Cerner order as well as the patient specimen cup.

## Enter patient’s First and Last name in the appropriate field on the Patient Data Screen. Click APPLY to save.

## Enter ordering doctor and select name of person performing the test.

## Select name of CLS releasing results.

## Attach a video to the report. Ensure the testing capillary is in the visualization (upper) chamber of the SQA-V analyzer. Click the PREVIEW VIDEO button on the V-Sperm Patient Data Screen.

## Turn the focus knob on the SQA-V analyzer to focus the image. If motile sperm are present in the sample, try to focus on the motile sperm. Click CAPTURE VIDEO to attach a live video to the patient record.

## Click the STOP CAPTURING button to stop the process after approximately 5 seconds. A message will indicate Video Saved. Click OK to end.

## **Microscopic Examination:**

## If motile sperm are detected by the SQA-V analyzer either by the automated or visualization system, testing is complete. No manual microscopic examination is needed.

## If no sperm or only immotile sperm are detected by the automated or visualization portions of the SQA-V analysis, a careful microscopic examination of the sample is needed.

## **NOTE:** Do NOT centrifuge post vasectomy samples.

## Thoroughly mix the sample by aspirating the sample 10 times into a disposable plastic pipette. Do not introduce air bubbles. Do not mix with a vortex.

## Place one 10 µl aliquot of semen on each of 2 slides and place under 22 mm x 22 mm coverslips.

## Examine the slides with a lowered condenser using a 40x objective.

## Scan the entire coverslip, field by field in a zig-zag fashion in order to make a complete and systematic search of the entire aliquot.

## **NOTE:** This procedure involves scanning approximately 1000 fields per slide and should take a minimum of 10 minutes for each slide.

## Scan the entire coverslip of the second slide as described above.

## At any point if motile sperm are seen, the microscopic examination is complete.

## Record the results of the microscopic examination in the comment field of the V-Sperm report.

### Select Patient Data and then select Patient List.

### Select the appropriate patient record from the list.

### Click the Enter Data button.

### Click inside the Comment field and enter the results of the microscopic examination (i.e. Immotile sperm were present, but motile sperm were absent in the microscopic examination of the uncentrifuged sample).

## Print test report and retain report and Patient Instruction Form.

# Interpretation of Results:

## >30 motile sperm seen/detected by SQA-V analysis is equivalent to MSC (motile sperm concentration) >2M/ml. Samples with MSC >2M/ml should be run as a normal (FRESH) sample instead of a POSTVASECTOMY sample. The numerical MSC is reported.

## Samples with 1-30 motile sperm detected by the SQA-V visualization system or samples with motile sperm detected by manual microscopic examination have a MSC of <2M/ml.

## If no sperm are detected by the SQA-V analyzer and no sperm are seen in the microscopic examination of the uncentrifuged specimen, the presence of sperm is said to be below the laboratory’s limit of detection.

# Result Reporting:

## Enter the test accession number in **ACCESSION RESULT ENTRY**.

## Right click on the Post Vasectomy result field. Select SPERM SEEN or CONVERT RESULT and then select FREETEXT, and type ‘See Comment” if no sperm are seen

## Attach a Result Comment. Right click in the Result field and select COMMENT, or click the COMMENT ICON.

## The Comment box appears. Select EDIT to enter results.

**NOTE:** Enter the Motile Sperm Concentration result in the Result Comment if the sample contains sufficient motile sperm (>30) to test as a Fresh sample.

* 1. Each Post Vasectomy report must have a statement regarding the presence/absence of motile/immotile sperm identified during the SQA-V analysis.
	2. Select and add the appropriate result comment based on the patient’s SQA-V results.

### *No motile or immotile sperm identified by SQA-V analysis.*

### *Both motile AND immotile sperm identified by SQA-V analysis.*

### *Immotile sperm identified by SQA-V analysis, but NO motile sperm identified by SQA-V analysis.*

### *Motile sperm identified by SQA-V analysis, but NO immotile sperm identified by SQA-V analysis.*

## If no motile sperm are identified during the SQA-V analysis, each post vasectomy report must have a statement regarding the presence/absence of motile/immotile sperm seen in the microscopic examination of the uncentrifuged sample.

* 1. Select and add the appropriate comment based on the patient’s microscopic results.

### *No motile or immotile sperm were seen in the microscopic examination of the uncentrifuged sample. The presence of sperm is below the limit of detection.*

### *Both motile AND immotile sperm were seen in the microscopic examination of the uncentrifuged sample.*

### *Immotile sperm were present, but motile sperm were absent in the microscopic examination of the uncentrifuged sample.*

### *Immotile sperm were present, but immotile sperm were absent in the microscopic examination of the uncentrifuged sample.*

## Add the date and time.

## Review the Result Comment for accuracy. Select VERIFY to finalize the result.

# Limitation of Procedure:

## The absence of motile/immotile sperm from the aliquot examined microscopically does not necessarily mean that motile/immotile sperm are absent from the rest of the sample.

## The SQA-V post vasectomy analysis 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.

## Low quality test results may be reported as < or > by the SQA-V analyzer 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.

## 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 22-26 °C.

# References:

## SQA-V Gold User Guide, Version 2.60. Medical Electronic Systems, LLC. Los Angeles, CA, Revision July 15, 2013.

## V-Sperm Gold User Guide, Version 3.60. Medical Electronic Systems, LLC. Los Angeles, CA, Revision July 15, 2013.

## WHO laboratory manual for the Examination and processing of human semen, Fifth Edition. WHO Press, World Health Organization. Geneva, Switzerland, 2010.

## Collage of American Pathologists, Hematology and Coagulation Checklist. Northfield, IL, Current Addition.

## Urinalysis and Body fluids, Fifth Edition. Strasinger and Schaub Di Lorenzo, F.A. Davis Company. Philadelphia, PA, 2008.

## QwikChek™ Test Strip QC Kit Product Insert. January 2023.

## 7500.CC.67 SQA-V Gold Semen Analysis