Semen Analysis: SOP (Standard Operating Procedure)

LABORATORY TEST #: (enter)

SPECIMEN TYPE: Live Human Semen

REVISION DATE: June 16, 2014

INSTRUMENTATION: SQA-V Gold Semen Quality Analyzer

VENDOR: Medical Electronic Systems LLC

METHOD: Mixed Technology

DISCIPLINE: (enter)
CLINICAL SIGNIFICANCE

A semen analysis is performed in order to determine the fertility potential of a male. At a minimum, three basic parameters are assessed: Sperm concentration, motility and morphology. A semen analysis panel may include other parameters ordered by the M.D.

TEST PRINCIPLE

The SQA-V is an automated sperm quality analyzer. The system performs a highly reliable 70-second semen analysis that follows the WHO (3rd, 4th or 5th Edition) guidelines for analyzing Sperm Concentration, Motility, Normal Morphology and many other parameters. The SQA-V can test FRESH, FROZEN, WASHED and POST VASECTOMY sample types and it runs assayed QwikCheck-beads (latex) for QC purposes and stabilized sperm for proficiency testing.

In addition to automated testing, the SQA-V visualization system magnifies samples x300 to x500. Test results and patient data are transferred from the SQA-V testing system to a V-Sperm computer for data base management. Patient data, test data and additional semen testing results such as WBC’s, pH, volume, viscosity, liquefaction status, etc. can be entered into V-Sperm and included in one patient report. Semen pictures and video clips can be attached to patient records for documentation purposes.

QwikLink-LIS interface allows transfer to the laboratory LIS system.

SPECIMEN REQUIREMENTS

- Specimen type: Semen
- Specimen volume: Entire ejaculate
- Minimum volume: Approximately 0.5 ml or 0.3 ml (diluted 1:1)
- Maximum ejaculation to test time: 1 hour

Provide the patient with the instructions: Patient Instructions for Semen Collection (Refer to Appendix 1) and verify that they have followed these instructions summarized below:

- 2-7 days abstinence from ejaculation prior to specimen collection
- Collect sample by masturbation only
- Lubricants, spermicides and other contaminants are not to be used
- The entire specimen must be collected into a clean container (preferably supplied by the physician’s office or the laboratory)
- The specimen container should be clearly labeled with the patient name and identifying information
- Transport the specimen to the laboratory right after collection (if collected off-site)
- Keep the sample at room temperature during transportation. Do not heat or cool the sample or the container

The semen sample should be tested within one hour of collection. Semen samples must be tested by the laboratory on a priority basis upon delivery - expedite to the testing area.

The entire ejaculate is required for determining sample volume. The collection container should remain at room temperature until liquefaction is complete or 45 minutes, whichever is shorter. Testing must begin within 60 minutes of specimen collection because motility will decline.

Some samples will not liquefy within 45 minutes (most will liquefy within 15 minutes). If, after 45 minutes the sample has not liquefied, treat with one vial of powder from the QwikCheck Liquefaction kit, following the package insert instructions. If a specimen is not liquefied, the accuracy of the analysis will be compromised.

SAMPLE REJECTION CRITERIA: Specimens received greater than 2 hours after collection. If testing begins greater than 60 minutes but less than 2 hours after sample collection please note: Results questionable due to age of specimen. It is important to eliminate as many variables as possible when conducting semen analysis testing.
REAGENTS and SUPPLIES

- SQA-V GOLD Analyzer and V-Sperm GOLD software
- SQA-V Capillaries (Catalog # 0402) Medical Electronic Systems LLC
- SQA-V Cleaning Kit (Catalog # 0115) Medical Electronic Systems LLC
- SQA-V Printer Paper (Catalog # 0314) Medical Electronic Systems LLC
- SQA-V Printer Ribbon (Catalog # 0312) Medical Electronic Systems LLC
- Microscope slides, glass, 1” x 3”.
- Coverslips, 22 x 22 mm.
- Short-range pH indicator paper, (e.g., 6.0-8.0, Fisher Cat.No. 14-853-90).
- QwikCheck-Test Strips for Semen Analysis (Catalog # 0700) Medical Electronic Systems, LLC
- QwikCheck Liquefaction Kit (Catalog #0900) Medical Electronic Systems, LLC
- QwikCheck Dilution Kits (Catalog #0800) Medical Electronic Systems, LLC
- QwikCheck Beads (Catalog # 0200) Medical Electronic Systems, LLC
- SQA-V Cleaning Kit
- Vortex Mixer

CALIBRATION

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.
- During this time do not touch the system or insert a capillary for testing
- When the MAIN MENU appears, the SQA-V is ready for testing
- Turn on the V-Sperm computer at this time and run and log into V-Sperm.
- If the SQA-V GOLD requires i-button tests to be loaded, please see the appendix section of this document for instructions

QUALITY CONTROL

Standardizing semen analysis in the laboratory with an automated system will lead to greater testing accuracy and precision. Proficiency testing surveys (CAP Survey) and periodic intra-laboratory testing will further evaluate the accuracy and precision of the laboratory by comparing their performance in relation to a peer group.

QwikCheck™ beads is a control material assayed for the SQA-V and other sperm counting systems (for Concentration). It is for in-vitro use only and is used to assess the accuracy and precision of the SQA-V by providing a known target value and +/- range. A high and a low known concentration and one negative control (for POST VASECTOMY controls) are supplied in 5 ml aliquots. Store at room temperature (20-25 °C or 65-77 °F). The expiration date assumes that the beads are stored at room temperature in their original containers and tightly capped to prevent evaporation.

Basic instructions:

Run controls at the beginning of the shift prior to testing any patient samples.
- With each new box of QwikCheck-beads, follow the SQA-V User Guide instructions for setting up the defaults
- Before opening, thoroughly mix the QwikCheck-beads. 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.
- Open the beads and immediately withdraw a sample of the control material.
- Immediately and tightly close the container after use to avoid evaporation or spillage.
1. Select: **RUN CONTROLS** from the **MAIN MENU** of the SQA-V.

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

3. Press **ENTER** to continue

4. Follow the instructions for mixing and preparing QwikCheck-beads noted previously.

5. 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.

6. 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.

7. Testing will begin automatically.

8. Print and save Control test results.

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**PROCEDURE NOTES AND PRECAUTIONS**

Enter Patient and Sample Data into the SQA-V:

- From the **MAIN MENU** select: **TEST NEW PATIENT** and press **ENTER**

- **ENTER PATIENT/SAMPLE DATA** in the screen below.

```
     Enter Patient and Sample Data into the SQA-V:
     
     - From the MAIN MENU select: TEST NEW PATIENT and press ENTER

     MAIN MENU
     
     TEST NEW PATIENT
     RUN CONTROLS
     SERVICE

     - ENTER PATIENT/SAMPLE DATA in the screen below.

     ENTER PATIENT / SAMPLE DATA
     PATIENT ID:    5788114
     BIRTH DATE:   01/01/85
     ABSTINENCE:   4 DAYS
     SAMPLE PROCESSING
     SAMPLE / ACCESSION #: 55
     COLOCTED:        DD/MM/YY   HH:MM
     RECEIVED:        DD/MM/YY   HH:MM

     o Patient ID - unique number identifying the patient
     o Birth Date - Birth date of the patient
     o Abstinence - # of days since the patient's last ejaculation
     o Sample/Accession # - up to 20 numbers identifying the sample
     o Collected - the date and time the sample was collected
     o Received - the date and time the sample was received.

     - Press **ENTER** to view the SAMPLE TYPE screen:

     SAMPLE TYPE
     SELECT: FRESH / WASHED / FROZEN / POST/ASECTOMY
     VOLUME: 2.5 ml
     WBC CONC.: SELECT <= 1 Mill/ml OR > 1 Mill/ml
     PH: 7.3
     APPEARANCE: NORM/ABNORM.
     LIQUEFACTON: NORM/ABNORM.
     VISCOSITY: NORM/ABNORM.

     - Select: Sample Type (required entry) based on the following options only:
     o FRESH - samples tested within an hour of collection and not enriched, diluted or treated.
```
o **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).

o **WASHED and FROZEN** – To run these samples please see the User Guide for instructions

- Enter the following information:
  
  o **VOLUME** - **REQUIRED ENTRY** The volume of the whole ejaculate in milliliters
  
  o **WBC CONC** – **REQUIRED ENTRY** Select $\leq 1M/ml$ (normal) or $> 1M/ml$ (abnormal) leukocytes (see how to test for this below)
  
  o **PH** – pH of the semen sample

### QwikCheck™Test Strips for Semen Analysis Leucocytes and pH:

- Place one drop of semen on each of the two test patches
- Wait 60 seconds
- Compare leukocyte and pH results to the color chart provided on the product label
- **WBC's**: If $\geq 1M/ml$ (dark lavender) select Abnormal (Abnorm) in the Sample Data screen
- **pH**: Enter the number most closely associate with the color of the patch

o **Appearance** - NORM./ABNORM visual assessment of the specimen

o **Liquefaction** – NORM./ABNORM.(Norm - liquefies within 60 minutes @ room temperature), Enter: ABNORM. if QwikCheck Liquefaction Kit successfully liquefied the sample.

o **Viscosity** - NORM./ABNORM (Enter ABNORM. if the sample reached appropriate viscosity using QwikCheck Liquefaction Kit)

### QwikCheck™Liquefaction:

- Select one vial of QwikCheck liquefaction powder
- Tap the vial to move the contents to the bottom of the vial before opening
- Add the entire contents of one vial to a viscous semen sample
- Gently mix the sample to dissolve the powder
- Once the sample has liquefied (5-10 minutes) test in the SQA-V

- **Sample Volume**
  
  o Using the left/right arrow keys on the keypad of the SQA-V select:
    - **YES**, for NORMAL VOLUME samples $\geq 0.5ml$ - **ENTER** to select
    - **NO**, for LOW VOLUME samples $< 0.5ml$ - **ENTER** to select

### IS SAMPLE VOLUME SUFFICIENT FOR COMPLETE TESTING $\geq .5 ml$?

**YES/NO**

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

**NORMAL VOLUME SPECIMEN**

FILL, CLEAN AND WIPE CAPILLARY

**INSERT IN CHAMBER**
- Prepare a testing capillary for a normal volume sample (see Appendix section for instructions)
- When the AUTOCALIBRATION is finished (about 15 seconds) and the screen will say “INSERT INTO TESTING CHAMBER”:
  - **Recommended**: Before inserting testing capillary into testing chamber, 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 re-enter sample information as a “POSTVASECTOMY” Sample Type – this will ensure the greatest possible accuracy in sample analysis.
  - **Recommended**: Samples should also be observed for significant quantities of debris, uric acid crystallization, opaque or thick seminal plasma and/or other significant abnormalities. Such cases should be noted on the report and/or re-confirmed by thorough manual observation.
  - Insert the testing capillary in the LOWER measurement compartment of the SQA-V
  - Make sure the BLUE STOPPER of the capillary is pointing down and the capillary has been completely wiped free of sample before insertion.
  - 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
INSERT IN CHAMBER
```

**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 ONLY
MOTILITY PARAMETERS ONLY
```

- Select the option: DILUTE SEMEN 1:1 WITH MEDIA, ENTER
- 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
INSERT CAPILLARY INTO CHAMBER
```

**QwikCheck™ Dilution:**
- Measure the volume of the neat semen sample.
- Dilute 1:1 with QwikCheck-Dilution
- Thoroughly mix the sample in order to evenly distribute the spermatozoa throughout the sample without introducing bubbles (do not use a pipette to mix)
- 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.
TESTING: Once the capillary is inserted into the SQA-V testing compartment (with the blue stopper facing downwards), testing will begin automatically.

- The testing cycle takes about 70 seconds
- 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 < 5M/ml sperm or no motile cells)
- DO NOT TOUCH THE SYSTEM during the testing cycle.
- Insert the testing capillary in the UPPER visual compartment of the SQA-V to CONFIRM Test Results (ie. if you have a high motility make sure sperm are moving fast).

TEST RESULTS: The two screens below will display complete test results.

![TEST RESULTS Table]

- Test results will be automatically saved and printed by the SQA-V.
- The screen below will be displayed in order to transfer the test results to the V-Sperm computer.

TO TRANSFER TEST RESULTS TO V-SPERM:

PRESS: “IMPORT TEST” BUTTON
IN V-SPERM


CALCULATION OF RESULTS

No calculations required

REPORTING RESULTS

1. The SQA-V will automatically print and save the test results.
2. Transcribe printed results onto the worksheet.
3. Enter results from worksheet into the computer system
4. Release results from the computer system
5. Print released results using the computer system
6. Attach printed results to worksheet
**DAILY MAINTENANCE**

- Perform daily cleaning when semen samples are run. See appendix for cleaning procedure.

**DYNAMIC RANGE OF THE SQA-V GOLD**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>SPERM CONC in M/ml</th>
<th>MSC in M/ml</th>
<th>Motility %</th>
<th>Morphology %</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRESH</td>
<td>2-400 or &lt; 2 M/ml</td>
<td>0.2-400 or &lt;0.2 M/ml</td>
<td>0-100%</td>
<td>0-100%</td>
</tr>
<tr>
<td>WASHED</td>
<td>2-200 or &lt; 2 M/ml</td>
<td>0.2-200 or &lt;0.2 M/ml</td>
<td>0-100%</td>
<td>0-100%</td>
</tr>
<tr>
<td>FROZEN</td>
<td>Not reported</td>
<td>0.2-200 or &lt;0.2 M/ml</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>POSTVASECTOMY</td>
<td>Manual Input</td>
<td>0-30 Sperm/Scan</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

**SEmen PARAMETERS AND WHO RANGES REPORTED BY THE SQA-V GOLD**

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

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

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

2. Motility testing is time sensitive and is run FIRST on the SQA-V GOLD. Specimens received more than one hour, but less than two hours after collection should be analyzed. Report OLD2 with motility.

3. If there the semen sample is not sufficient for even LOW VOLUME testing (250 µl), append the abbreviation QNS to those tests that were not completed.

REFERENCES


3. Medical Electronic Systems LLC; SQA-V GOLD User Guide

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

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

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

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

8. Dr. Lev Rabinovich, Chief Technology Officer ( Medical Electronic Systems )
Appendix 1: Setting-up a NEW BOX of QwikCheck Beads CONTROLS

Set-Up: Assayed Control
Each time a new lot of QwikCheck-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.

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 GOLD on the PC and select: SET-UP > SQA-V > SQA-V Defaults and press CONTINUE.
Step 4: The set-up screen below will be activated in V-Sperm GOLD on the PC. Go to the Control section of the screen below:

Step 5: Select: Latex Beads
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: Click on APPLY - The set-up may take two minutes.
Appendix 2: Adding I-Button Tests

The SQA-V GOLD requires that I-button tests are loaded into the system in order to run a test. Please follow the instructions below to add tests:

1. Connect the SQA-V to the PC and then go to:
2. V-Sperm > Set-up > SQA-V > I-Button & follow the instructions
3. Go to the SQA-V > Main Menu > Service > Add I-Button tests
4. Insert I-button into port and follow screen instructions
5. Hold the I-button firmly against the port side, without moving it
6. Keep holding the I-button until #TESTS ADDED is displayed

*For SQA-V versions prior to GOLD follow instructions 3-6 only
Appendix 3: Loading the SQA-V Testing Capillary

Filling the SQA-V Capillary with a Normal Volume Sample

Before filing 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.

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.**

Appendix 4: Using the SQA-V Visualization System
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.
- To view a slide in the slide adaptor, prepare a slide as follows:
  - 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.

To view the SQA-V testing capillary in the visualization system:

- Fill the SQA-V testing capillary (see Appendix) and insert the testing capillary with the BLUE STOPPER facing upwards (the opposite of when you are testing).

Visualization Process:

- The video display will automatically illuminate when the SQA-V is turned on.
- 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.
  - Turn BRIGHTNESS, CONTRAST and COLOR buttons all the way counterclockwise.
  - Turn FOCUS knob fully clockwise.
  - 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.
- Turn BRIGHTNESS knob clockwise until the video screen begins to lighten-up.
- Turn FOCUS knob counterclockwise until image is focused.
- 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:

- Insert standard slide (10 µl sample, 22X22 mm coverslip) into the visualization compartment.
- Press ZOOM OUT (x300) all the way.
- Bring the image into focus.
- The screen is divided into a 20-square "grid" in order to make counting easier for the user.
- Each sperm cell seen on the ENTIRE screen represents 1 Million/ml.
Appendix 5: SQA-V Cleaning Instructions

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 contents:
- Long cleaning brush
- Blue “dot” cleaning capillaries
- Sponge drying capillaries
- Cleaning fluid

Cleaning: Step 1 (DAILY)
1. Insert the long brush (bristle-side down) fully into the upper portion of the lower chamber of the system in the same manner as a testing capillary (Fig. 1).

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)

3. Monitor the systems “REF. 2” parameter. It should be between 2,800 and 3,200 mV if possible.

Cleaning: Step 2 (WEEKLY)
1. Select a Blue dot cleaning capillary (Fig.4)
   - Moisten with ONE drop of cleaning fluid
   - Shake off excess fluid
   - Insert into measurement compartment with blue dot and fibrous and material facing DOWN ONLY (Fig.5)
   - Move cleaning capillary in and out three times.

2. Insert the sponge-tipped drying capillary into the testing chamber and leave it for 10 seconds (Fig.6)

NOTE: Do not move this drying capillary in and out.
PROCEDURE TITLE: Semen Analysis, Basic

APPROVAL AND REVIEW

PREPARED BY: ____________________________

DATE: ____________________________

DISCIPLINE DIRECTOR APPROVAL: ____________________________

DATE: ____________________________

DEPARTMENTAL DIRECTOR (or DESIGNEE) APPROVAL (at site):

DATE: ____________________________

IMPLEMENTATION DATE (at site):

DATE: ____________________________

SUPERSEDES SOP DATED: ____________________________

DATE SOP RETIRED (at site): ____________________________

DEPARTMENTAL DIRECTOR (OR DESIGNEE) ANNUAL REVIEW:

<table>
<thead>
<tr>
<th>SIGNATURE</th>
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