MEDICAL ELECTRONIC SYSTEMS
5757 W. Century Blvd. Suite 805
Los Angeles, CA. 90045
"Remember, it ALL Started with a Sperm!"
www.mes-global.com

SQA-V Gold Semiannual Validation/Proficiency/QC Recommendations

OVERVIEW

Providing the best patient care requires laboratory staff and pathologists to strive for the highest levels of performance. In order to meet these standards, maintain user proficiency, and confirm the correct operation of the SQA-V Gold Automated Sperm Quality Analyzer, MES had developed a series of recommendations to be performed on a semiannual basis. These recommendations include:

1. Semiannual System Mini-Validation and Motility/Morphology QC

It is highly recommended that the facility confirm the precision and lower limit detection ability of the SQA-V Gold by completing an abbreviated validation study on a semiannual basis. It is also suggested that 5 samples be compared to the backup method on an annual or semi-annual basis (at the laboratories discretion). In addition, organizations providing semen analysis proficiency testing programs (CAP, NEQAS) require laboratories to show proficiency and quality control across three main semen analysis parameters: Sperm concentration, motility and morphology. To date, the QC/proficiency testing samples provided by these organizations do not address motility or morphology due to natural limitations associated with shipping live samples. This recommended Mini-Validation in conjunction with the SQA-V's daily calibration/self-test, and daily zero level MSC confirmation can be used to demonstrate ongoing motility and morphology quality control. For additional motility/morphology QC information please contact MES directly to request a technical bulletin (service@mes-llc.com).

2. Semiannual Instrument Calibration Confirmation

It is recommended that twice per year, the SQA-V Gold calibration is checked against the original factory calibration parameters. Although there are acceptable calibration ranges for the SQA-V, the system parameters may be close to the high or low end of the range and proactive maintenance will ensure continued uninterrupted use and optimal clinical performance from the instrument.

3. Semiannual College of American Pathologists (CAP) Proficiency Challenge

The SQA-V Gold is part of the CAP semen analysis proficiency challenge and as an automated method has a specific sample set that is peer reviewed against other SQA-V Gold users. MES recommends this survey as an unbiased appraisal of user proficiency and system performance. Please contact CAP directly for more information and for ordering details: http://www.cap.org/apps/cap.portal.

Manufacturers Validation Kit (OPTIONAL)

MES Manufacturers an optional "Validation Kit" designed as a proficiency, training, and validation tool for the SQA-V Gold. It can be used to validate Concentration Accuracy, Precision, Lower Limit Detection and Reportable Range (Linearity) per CLIA Method Validation Regulations. Product Code: A-CA-00691-00 / www.mes-global.com/sqa-validation-kit.

SYSTEM MINI-VALIDATION AND MOTILITY / MORPHOLOGY QC

SETUP

- Turn the SQA-V Gold system and allow the instrument to complete its Stabilization and Self-Test.

- Print and save the system's Service Data Report.

- Check the Service Data Report to verify that the REF. 2 parameter is between 2,800 and 3,200 (ideal range).
  - If this value is below 2,800 mV or above 3,200, perform a thorough cleaning of the system per user guide instructions.

- Save all printouts processed during the validation. Return your data to MES via Fax or E-mail for crunching (310-670-9069 / service@mes-llc.com).

PRECISION AND SENSITIVITY

Running 5 replicate determinations on at least two levels of live human sperm is recommended to estimate the imprecision or random error of the method. For the detection limit experiment, a "blank" material is analyzed in 5 replicates. MES QwikCheck Beads negative control is recommended for the lower limit detection portion of the experiment.

FOR LIVE HUMAN SAMPLES:

- NOTE: Do not load a new capillary or discharge and refill the capillary between replicate tests; re-run the same aliquot in the same capillary for all 5 replicates.

- Run each of the 5 replicate semen samples through the "Test New Patient" option.

- For "Patient ID" use any sample number desired - No other data entry is necessary on the first screen.

- Choose "Fresh" as the sample type.

- Select <= 1 M/ml for the "WBC Conc." entry field.

- You may skip all other fields by pressing enter to advance to the Auto-Calibration screen (do not touch system during auto-calibration).

- Mix your sample (thoroughly) by aspirating the sample in and out 10 times with a transfer pipette.

- Load the testing capillary per the instructions in the SQA-V Gold User Guide.

  - Note: Take great care to avoid bubbles, under filling, or capillary tip "wicking" (during capillary drying).

- Insert capillary into the SQA-V Gold measurement compartment when prompted to do so.

- After the test is completed, import the result to V-Sperm and record Sperm Concentration, Motility and Morphology on the Mini-Validation Data Entry Sheet.

- Select "Retest Same Patient" after each test to run the next replicate.

  - Note: Time is of the essence. Minimize the delay between tests as much as possible.

- Enter all results into the SQA-V Validation Study Data Entry Sheet and return to MES for crunching.

- Repeat the steps above for all replicates across both live human sperm samples.

FOR NEGATIVE CONTROL ZERO LEVEL:

- Set the SQA-V Controls via the V-Sperm (See SQA-V Gold User for reference).

- Run 5 replicates of the negative control from the **Run Controls** section of the main menu. Choose the "Negative Control" option from the Run Controls menu (do not run as "level 1" or "level 2").

- Do not discharge and refill the capillary between tests; re-run the <u>same</u> aliquot in the <u>same</u> capillary.

- The control results are printed automatically.

- Enter CONC. and MSC values in the Semiannual Validation Study Results spreadsheet.

- Save all of the printouts and return them to Medical Electronic Systems for your final Validation Report.

ACCURACY

On an annual or semi-annual basis (at the users discretion) MES suggests comparing 5 patient specimens analyzed on the SQA-V Gold to the backup method to confirm user proficiency and correct system operation. MES suggests running 5 selected patient semen samples representing high, medium and low sperm concentrations. NOTE: Samples can be diluted and combined to achieve target qualities and increase the number of samples.

To achieve an accurate comparison, please review these requirements and recommendations closely before beginning:

- The SQA-V analyzes semen samples strictly according to WHO Manual criteria and accurate validation will be difficult if procedures are not followed closely by manual analysis. Please contact MES to request a copy of this manual if you don't have one. service@mes-llc.com. MES currently recommends WHO 5th edition criteria for manual analysis.

- Everything must be run at ROOM TEMPRATURE by both methods. Samples should never be incubated.

- All samples should be run within 1 hour of collection – <u>no exceptions</u> especially during validation. Samples should be run on the SQA-V first as the testing cycle is much shorter. Manually, Motility should be run first under the microscope to decrease any time variables.

- All samples need to be fully liquefied, and well mixed. Use QwikCheck Liquefaction material on stubborn, viscous, and agglutinated or aggregated samples. This is a critical point for both accurate concentration and motility comparisons.

- We recommend visualizing all samples before testing on the SQA-V. Extreme "odd ball" cases to watch out for include large amounts of uric acid crystals or cell debris. Also, confirm that sperm are present – if you observe no or very few sperm, the sample should be run on post vasectomy mode or simply reported as "zero" across the board.

- When counting fields manually <u>do not ignore agglutinated or aggregated sperm</u>. The system counts all sperm, so if you ignore the large clumps you will affect the accuracy. This is a common mistake with manual analysis – there is a tendency to ignore fields that are clumped and agglutinated in search of "easier" fields to count.

- The SQA-V Gold analyzes Morphology according to Kruger STRICT criteria under WHO 5th edition reference ranges. It is critically important that STRICT criteria be followed by manual observation. For clarification of these methods please contact MES directly for support: service@mes-llc.com.

- It is common to overestimate sperm motility (manually), but this can often be avoided by reversing the order of analysis (NP and IM first), using an eyepiece reticle, and being aware of, and avoiding, to the extent possible, potential sources of bias.

- Make the preparation immediately before assessing. Read only after any drifting has stopped to reduce bias in overall motility.

- Select the field randomly and do not deliberately select fields with high or low numbers of motile spermatozoa. One way to do this is to avoid looking through the oculars until a field has been selected.

- Do not wait for motile spermatozoa to enter the field before starting to count.

- Analyze quickly; analyze only a small portion of the grid at one time, depending on sperm concentration.

- Spend less time examining one area of the grid, to avoid counting spermatozoa that swim into the area during analysis.

- Count progressive, non-progressive and immotile spermatozoa in two stages. If there are problems with the technique, reverse the order of analysis (Review Table 7.4 below):

Table 7.4 Sources of variation (error) in assessing sperm motility and proposed solutions

Procedure	Prevention	Control
Improper mixing of specimen before aliquot is removed	Training, SOP	Replicate sampling and assessment, IQC
Waiting too long after slide is prepared before analysis (spermatozoa quickly lose vigour)	Training, SOP	Replicate sampling and assessment, IQC
Improper temperature of stage warmer (e.g. too high temperature will kill spermatozoa)	Training, SOP, equipment maintenance	IQC
Microscope not properly cleaned or aligned. Improper magnification	Training, SOP, equipment maintenance	IQC, EQC
Lack of eyepiece grid for guidance	Equipment	IQC (control chart)
Analysing around the edges of the coverslip (the spermatozoa die or become sluggish around the outer 5mm of the coverslip)	Training, SOP	Replicate assessment, IQC
Making the assessment too slowly (other spermatozoa swim into the defined area during the assessment period)	Training, SOP	IQC
Malfunction of multikey counter	Equipment maintenance	IQC, EQC
Errors in calculating percentages if not counted in multiples of 100	Training, SOP	IQC, EQC
Subjective bias (i.e. consistently too high % motile or too low % motile)	Training, SOP	IQC, EQC
Preparative procedures that reduce motility (e.g. temperature change, vigorous mixing, contamination with toxins)	SOP	IQC
Non-random selection of fields for analysis. Delay in analysis (e.g. waiting until motile spermatozoa swim into the field or grid to begin analysis)	Training, SOP	IQC, EQC

SQA-V ACCURACY TESTING INSTRUCTIONS

- Run each of the 5 semen samples through the "Test New Patient" mode.

- Enter your relevant patient and sample data in the available fields

- Choose a sample type.

    o Note: Azoospermic or extremely poor quality samples should be run on the Post Vasectomy mode.
    o Note: Extremely decreased viscosity (watery) sample should be run on the system's Washed mode.

- Select <= 1 M/ml or > 1 M/ml for "WBC Conc." based on results of QwikCheck Test Strips (see SQA-V Gold User Guide for reference).

- Select > 1 M/ml for samples with high amounts of debris or round cells (50%+).

- For short samples, you may choose a low volume option by selecting NO when asked if sample is greater than 0.5 mL.

- Mix your sample (thoroughly) by aspirating it in and out 10 times using a transfer pipette.

- Load the testing capillary per the instructions in the SQA-V Gold User Guide.

- Visualize the sample to confirm the presence of sperm, and to check for debris / round cells.

    o If no or few sperm are seen hit the Escape button twice and choose the Post Vasectomy testing mode.
    o If large amounts of debris or round cells (50%+) are seen, hit Escape twice and choose the > 1 M/ml option.

- After the test is complete, import your test into V-Sperm per onscreen instructions to archive the result.

- Perform manual testing per you Laboratories standard operating procedure.

    o **Note:** Please follow the recommendations from section 1 closely during manual analysis.

- Enter concentration, motility and morphology results from both methods into the SQA-V Validation Study Data Entry Sheet and return to MES for crunching.

INSTRUMENT CALIBRATION CONFIRMATION

CALIBRATION CONFIRMATION INSTRUCTIONS

Twice per year, MES recommends sending us the "Service Data Report" of your SQA-V Gold for a calibration confirmation. To print your Service Data Report and return it to MES, please follow these instructions:

- NOTE: The SQA-V internal printer will need to be loaded with paper and printer ribbon. Please see user guide for paper and ribbon loading instructions.

- Power your SQA-V instrument off, then on again so a fresh calibration is performed by the system. If the Self-Test data report does not print out automatically, navigate to the "Service" menu and once in the "Service" menu select "Print Default Settings". Then select "Print Self-Test Data".

- Tape this Self-Test report to a piece of blank white paper and fax the report to MES @ 310-670-9069 or scan and e-mail it to service@mes-llc.com. Please include an e-mail address and your contact information with the Self-Test report.

- Your SQA-V calibration parameters will be compared to the initial calibration parameters from when the instrument was manufactured and presented back to you in report format

COLLEGE OF AMERICAN PATHOLOGISTS (CAP) PROFICIENCY CHALLENGE

CAP SAMPLE PROCESSING INSTRUCTIONS

NOTE: The CAP proficiency material is run as a CONTROL not as a patient sample.

- Turn on both the SQA-V and the PC loaded with V-Sperm software.
- From the main menu of the SQA-V select: SERVICE > SERVICE DATA.
- From the V-Sperm computer, navigate to SET UP > SQA-V > SQA-V DEFAULTS and click "CONTINUE".
- On the bottom half of the Setup screen, find "CONTROLS". Select the STABILIZED SPERM option, and enter the following for Level 1 and Level 2:
    o LOT NUMBER: Your CAP reference number.
    o TARGET VALUE: Enter 50 for all levels.
    o RANGE: Enter 50 - DATE: Today's date.
- Click "APPLY" and wait a few moments for the information to transfer to the SQA-V. Once the transfer is complete you are ready to run the Proficiency Test on the SQA-V Gold.

Running Stabilized Sperm CAP Proficiency Material on the SQA-V:

- From the MAIN MENU of the SQA-V select: RUN CONTROLS > LEVEL 1
- Load the SQA-V testing capillary and insert it into the testing chamber per the onscreen instructions.
- If you receive a result of 0.0, Re-Run the sample on the SQA-V's "Test New Patient" FRESH mode (see Note below).
- Repeat this process for Level 2.
- Record your actual TEST results and submit them to CAP.
- Please contact MES with any questions at service@mes-llc.com or 886-557-9064.
- Remember to re-set your control defaults back to Latex Beads after completing the proficiency challenge!

NOTE: Due to the nature of prepared "Stabilized Sperm" proficiency material, very low concentration samples may report "ZERO" even though a few sperm a present. This occurs because the optical density of the Stabilized Sperm may be below the dynamic range of the instrument. If you receive a "ZERO" result on the QC mode described above, it is recommended that you re-run the sample on the SQA-V's FRESH mode to achieve and report a result of < 2.0 M/mL. For clarification please contact MES directly.