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“Remember, it ALL Started with a Sperm!”

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Medical Electronic Systems SQA-V Gold Validation Recommendations

OVERVIEW

This Medical Electronic Systems Validation Recommendation template was designed to help validate the Accuracy, Precision and Reportable Range of the SQA-V Gold sperm quality analyzer per the New CLIA Method Validation Regulations (CLIA Final Rules Manual, 2004, ISBN 1-886958-20-3). The regulations disseminated on February 28, 1992 for laboratories to comply with the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88) included specific quality control (QC) regulations for laboratories performing moderate and high complexity testing. These regulations also contained specific method validation requirements for modified moderate and high complexity tests and tests developed in-house. Test sites performing unmodified, FDA approved, moderate complexity testing could accept the manufacturer's performance specifications and were not required to perform any method validation. On January 24, 2003, the Centers of Medicare and Medicaid Services (CMS) issued the final CLIA rules (CLIA Final Rules Manual, 2004, ISBN 1-886958-20-3). These rules bring ALL non-waived (moderate and high complexity) testing under uniform QC requirements, including validation of methods. Minimum QC now is two levels per day rather than per run. The approach in method validation is to perform a series of experiments designed to estimate certain types of analytical errors, e.g., a linearity experiment to determine reportable range, a replication experiment to estimate imprecision or random error, a comparison of methods experiment to estimate inaccuracy or systematic error, and a detection limit experiment to characterize analytical sensitivity. These SQA-V Gold Validation Instructions provide recommendations for validation of the SQA-V Gold system per CLIA 2003 (USA) standards for non-waived systems. After completion of this validation, your SQA-V Gold instrument will be ready to use in daily operation based on Medical Electronic Systems manufacturer requirements for instrument validation. Depending on your State and Local requirements further validation may be required beyond these recommendations so please check with your local regulatory agencies.

→ MES MANUFACTURERS VALIDATION KIT

Medical Electronic System's Manufacturers a "Validation Kit" designed as a proficiency, training, and validation tool for the SQA-V Gold sperm quality analyzer. It can be used to validate Concentration Accuracy, Precision, Lower Limit Detection and Reportable Range (Linearity) per CLIA Method Validation Regulations. Due to the fragile biological nature of sperm, parameters influenced by motion have been omitted from the Manufacturers Validation Kit. Supplemental recommendations and instructions for successfully validating Motility, and other motion based parameters are included with the kit. After successfully completing the MES Manufacturers Validation Kit users will have extensive knowledge of the SQA-V's operation, capillary loading technique, reporting, and more. In addition, annual or semi-annual personnel re-testing using the Validation Kit will demonstrate ongoing operator proficiency and the laboratory's commitment to accuracy, precision, and quality of care. Product Code: A-CA-00691-00 / mes-global.com/sqa-validation-kit. For additional information or to order this product please contact MES: Phone: 310-670-9066 / sales@mes-llc.com.

SQA-V Gold Initial Validation Instructions

SETUP

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

1. COMPARISON OF METHODS EXPERIMENT (ACCURACY) OVERVIEW

MES recommends running a minimum of 20 patient specimens by the new method (test method) and an established method (comparison method) to estimate the inaccuracy or systematic error of the method. At the same time, it is stated that the actual number of specimens tested is less important than the quality of those specimens. Twenty specimens that are carefully selected on the basis of their observed concentrations will likely provide better information than the a hundred specimens that are randomly received by the laboratory. The quality of the experiment and the estimates of systematic errors will depend more on getting a wide range of test results than a large number of test results.

Based on these comments, MES recommends running 20 selected patient semen samples representing high, medium and low sperm concentrations using the SQA-V Gold and a manual method. NOTE: Samples can be diluted and combined to achieve target qualities and increase the number of samples. Set the SQA-V Gold Concentration Standard according to your manual method (see SQA-V Gold User Guide for reference).

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

- 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 – no exceptions 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 do not ignore agglutinated or aggregated sperm. 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

2. INSTRUCTIONS FOR COMPARISON OF METHODS EXPERIMENT (ACCURACY)

- Run each of the 20 semen samples through the "Test New Patient" mode.
- Enter your relevant patient and sample data in the available fields
- Choose a sample type.
 - Note: Azoospermic or extremely poor quality samples should be run on the Post Vasectomy mode.
 - 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 sufficient for complete testing.
- 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.
 - If no or few sperm are seen hit the Escape button twice and choose the Post Vasectomy testing mode.
 - 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.
 - **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.

3. REPLICATION & DETECTION LIMIT EXPERIMENT (PRECISION)

Running a minimum of 10 replicate determinations on at least two levels of control materials is recommended to estimate the imprecision or random error of the method. For the Detection Limit Experiment, a "blank" material is analyzed in 10 replicates. MES QwikCheck Beads (Positive Levels and Negative Control) are recommended for this experiment. Two levels of QwikCheck Beads (1 and 2) and a Negative Control ("blank") should be run in 10 replicates.

- Set the SQA-V Control reference ranges via the V-Sperm setup menu (See SQA-V Gold User for reference).
- Run 10 replicates of two bead levels from the Run Controls section of the SQA-V's main menu. Do not discharge and refill the capillary between tests; re-run the same aliquot in the same capillary.
- Run 10 replicates of the Negative Control. NOTE: Make sure you choose the "Negative Control" level (not level 1 or 2). Record both the Concentration and MSC results on the data entry spreadsheet.
- The control results are printed automatically.
- Enter all control results into the SQA-V Validation Study Data Entry Sheet and return to MES for crunching.

It is also suggested that at least 2 Live Patient Samples be run in replicates of 5 each to establish precision for Motility and Morphology results.

- 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.
- Run 5 replicates of each live human sample from the Test New Patient section of the SQA-V's main menu. Do not discharge and refill the capillary between tests; re-run the same aliquot in the same capillary.
- 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.

4. LINEARITY & REPORTABLE RANGE EXPERIMENT

Centrifuge multiple semen samples and decant the supernatant to achieve a sample concentration of at least 400 M/ml in 3 ML of total volume (1,200 million sperm total). Load and run all samples in the proportions below on the SQA-V's Fresh testing mode.

- Prepare six samples by dilution of the Pooled semen sample using MES QwikCheck Dilution media in the following proportions: **100/0, 80/20, 60/40, 40/60, 20/80 and 0/100.**
- **First level:** Run concentrated specimen on "Fresh Mode" to establish the upper range of the linearity curve. Record the results on the data entry sheet.
- **Second level:** Mix 0.8 mL from the First Level and 0.2 mL of QwikCheck Diluent. This will create an 80/20 dilution. Run and record results on the data entry sheet.
- **Third level:** Mix 0.6 mL from the First Level and 0.4 mL of QwikCheck Diluent. This will create a 60/40 dilution. Run and record results on the data entry sheet.
- **Fourth level:** Mix 0.4 mL from First Level and 0.6 mL of QwikCheck Diluent. This will create a 40/60 dilution. Run and record results on the data entry sheet.
- **Fifth level:** Mix 0.2 mL from First Level and 0.8 mL of QwikCheck Diluent. This will create a 20/80 dilution. Run and record results on the data entry sheet.
- **Sixth level:** Run QwikCheck Dilution media as is for a 0/100 dilution. Run and record results on the data entry sheet.
- Note: Run these samples on the Test New Patient mode.
- Select "Fresh" for the sample type, and "<1M/mL WBC Concentration".
- Enter all results into the SQA-V Validation Study Data Entry Sheet and return to MES for crunching.

CONCLUSION

When all tests are completed, e-mail your data to: service@mes-llc.com, or fax it to 310-670-9069 ATTN: Validation. Your results will be compiled and presented to you within 5 business days. ***"Remember, it ALL Started with a Sperm!"***