MEDICAL ELECTRONIC SYSTEMS
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"Remember, it ALL Started with a Sperm!"
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Medical Electronic Systems SQA-VISION 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-VISION Validation Instructions provide recommendations for validation of the SQA-VISION per CLIA 2003 (USA) standards for non-waived systems. After completion of this validation, your SQA-VISION 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 line of 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-VISION'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-VISION Validation Instructions

SETUP

- Power on the SQA-VISION testing unit and PC then allow the calibration and self-test to complete.
- Confirm that all Key Parameters are passing and in range.
- Recommended Test Patient Settings:
    - CONC. Standard = 2
    - LES = ROW
    - Low Quality Counter = Checked
    - Debris / Round Cell Scan Cutoff = No Debris Scanning
    - Testing Criteria = WHO 5th (unless the current method is based on a different WHO version)
    - For assistance setting the defaults, please refer to the Help Menu of the Vision, the User Guide, or contact MES: (866-557-9064 / 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-VISION and a manual or backup method. NOTE: Samples can be diluted and combined to achieve target qualities and increase the number of observable samples.

NOTE: Results may be sent to MES for analysis (service@mes-llc.com). It is recommended that you send the first 5 comparison samples for an initial overview before completing the remainder of the validation. This will allow for any necessary troubleshooting or modification of technique. A data entry sheet is also available from MES upon request.

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

- The SQA-VISION analyzes semen samples strictly according to WHO Manual criteria and accurate validation will be difficult if WHO recommendations are not followed closely by the manual or backup method. Please contact MES to request a free copy of the WHO Manual for Semen Analysis if you don't already 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, pre heated or tested on a heated stage.
- All samples should be run within 1 hour of collection – no exceptions during validation. Samples should be run on the SQA-VISION first as the testing cycle is much faster. Manually, motility should be run first to decrease associated time variables.
- All samples need to be fully liquefied and well mixed. Use the QwikCheck Liquefaction Kit on stubborn, viscous, and agglutinated / aggregated samples. This is a critical point for both accurate concentration and motility comparison.
- After the automated testing cycle, samples should be visualized using the "Debris" scanning option on the SQA-VISION results screen. Select the debris % according to the instructions and options provided in the Debris Scanning interface.

- When counting fields manually do not ignore agglutinated or aggregated sperm. The system counts all sperm, so if you ignore the large clumps it will affect the comparison. 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-VISION analyzes Morphology according to WHO 3rd, 4th or 5th edition criteria. It is critically important that WHO standards be followed for the Manual or backup method as well. For clarification or questions on WHO criteria, 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 (Non Progressive and Immotile first), using an eyepiece reticle, and being aware of, and avoiding, to the extent possible, potential sources of bias **(see Section 7.13.3) – WHO 5th ed. manual, p. 24).**

- **Section 7.13.3 (WHO 5th ed. manual, p. 200-201)** Practical hints when experiencing difficulty assessing motility:

1. Make the preparation immediately before assessing. Read only after any drifting has stopped to reduce bias in overall motility.
2. Select the field randomly and do not deliberately select fields with high or low numbers of motile spermatozoa. NOTE: One way to do this is to avoid looking through the oculars until a field has been selected.
3. Do not wait for motile spermatozoa to enter the field before starting to count.
4. Analyze quickly; analyze only a small portion of the grid at one time, depending on sperm concentration.
5. Spend less time examining one area of the grid, to avoid counting spermatozoa that swim into the area during analysis.
6. 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. REPLICATION & DETECTION LIMIT EXPERIMENT (PRECISION)

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

- Set the SQA-VISION control information in the Settings section.
- Run 10 replicates of two bead levels from the QC / Proficiency section of the 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 1 or 2). Record both the Concentration and MSC results on the data entry spreadsheet.

Live Samples - It is also recommended that 2 live samples be run on the systems Fresh “Test Patient” mode to observe Motility and Morphology precision. NOTE: Only 5 replicates of each sample should be run (using the same aliquot in the same capillary). Sample stability may be effected by the time gap associated with running 10 replicates.

- Select “Fresh” from the Test Patient tab. Enter the required sample information and test the sample. On the results screen you will see the option to “Re-Test”. Choose this option to avoid delays between replicates.
- Record your results and return them to MES for analysis.

3. 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 on Fresh mode according to standard SQA-VISION testing procedures

- Prepare six samples by diluting 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. NOTE: Target is 400 M/mL but a result close to that is acceptable.
- **Second level:** Mix 0.8 mL from First Level and 0.2 mL of QwikCheck Diluent. This will create an 80/20 dilution. Run and record results.
- **Third level:** Mix 0.6 mL from First Level and 0.4 mL of QwikCheck Diluent. This will create a 60/40 dilution. Run and record results.
- **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.
- **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.
- **Sixth level**: Run QwikCheck Dilution media as is for a 0/100 dilution. Run and record results on the enclosed data sheet.
- Record all results and return to MES for analysis.

CONCLUSION – Results may be returned to MES for analysis at any time: service@mes-llc.com