



QwikCheck™ Beads Precision and Linearity Kit Instructions

QwikCheck GOLD Analyzer

OVERVIEW

The QwikCheck™ Beads Precision and Linearity Kit is designed as a semi-annual validation tool for QwikCheck GOLD, SQA-V and SQA-VISION sperm quality analyzers. It can be used to validate Linearity, Precision and Concentration Accuracy per the 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) and Validation regulations for laboratories performing moderate and high complexity testing. These regulations also contain specific method validation requirements for modified moderate and high complexity tests and tests developed in-house. The approach in method validation is to perform a series of experiments designed to estimate certain types of analytical errors: A linearity trial to determine the system response to sequential dilutions covering the device's reportable range; a replication trial to estimate imprecision or random error; a detection limit to characterize analytical sensitivity; a comparison of the automated to the manual method vs. pre-assayed target ranges to estimate inaccuracies or systematic errors for each method and assess the agreement between two. After successfully completing this validation, the system users will have satisfied the semi-annual system validation recommendation from Medical Electronic Systems. Depending on State and Local requirements, further validation may be required.

The pre-assayed target ranges of the samples in this kit will not be known to the user (blind study), but will be applied for both automated and manual assessments to demonstrate agreement between the methods. The instructions are broken into three documents: for **QwikCheck GOLD**, **SQA-V** and **SQA-VISION**.

The tests contained in this kit should all be run following best laboratory practices – samples should be thoroughly swirled for mixing prior to capillary aspiration, quality calibrated pipettes should be used, and all manual analysis techniques should follow WHO recommended protocols.

SYSTEM SET-UP and GENERAL INFORMATION

- After powering on the QwikCheck GOLD analyzer, print a copy of the Service Data report.
- Check the report to verify the Reference 2 (REF 2) value is within the recommended range of 2,800 to 3,200 mV.
- Set-up the QwikCheck GOLD default settings as described below.
- From the QwikCheck GOLD MAIN MENU select:
SERVICE > SET-UP > SYSTEM DEFAULTS. The following settings should be selected for the QwikCheck™ Beads Precision and Linearity Kit (you may return them to your specific settings afterwards):
 - ✓ DATE FORMAT
 - ✓ DATE/TIME SETTING
 - ✓ AUTO PRINTING: YES
 - ✓ # LABELS TO PRINT: 1
 - ✓ CONC. STD: 2
- From the QwikCheck GOLD MAIN MENU select:
SERVICE > SET-UP > CONTROLS. The following settings should be selected for the QwikCheck™ Beads Precision and Linearity Kit (you may return them to your specific settings afterwards):
 - ✓ Controls: LATEX BEADS
 - ✓ LEVEL 1 Target and Range = 100 +/- 99.9
 - ✓ LEVEL 2 Target and Range = 100 +/- 99.9
 - ✓ NEGATIVE CONTROL = 0.0

STEP 1: QWIKCHECK GOLD LINEARITY & REPORTABLE RANGE PROTOCOL

NOTE: Before opening each sample bottle, swirl the beads 10~15 times in each direction for proper mixing.

NOTE: Run LINEARITY & REPORTABLE RANGE samples on the QwikCheck GOLD **TEST NEW PATIENT** mode.

- QwikCheck GOLD LINEARITY & REPORTABLE RANGE Sample Testing and Reporting:
 - From the MAIN MENU select: TEST NEW PATIENT.
 - Enter only the mandatory information below (you may skip all other fields):
 - Patient ID: Use the sample # on the top of each testing bottle.
 - Sample Type: FRESH.
 - WBC CONC: < 1 M/ml.
 - Continue pressing ENTER to advance to the Auto-Calibration screen.
 - Wait for Auto-Calibration to complete without touching the system and then run the testing capillary.
 - Record your results.

NOTE: To run this protocol use the six (6) **Blue Lettered “Linearity”** bottles marked “1” through “5” and the bottle marked “D”. Using a quality calibrated pipette, make serial dilutions from bottle “1” by following the instructions below. Record results on the data sheet provided in the kit or in the Validation Data Entry form (Excel). The message: “Low Quality Sample testing will take 2 more minutes” may be displayed during some tests. This is normal and occurs because there is no motility present.

- **First level:** Run bottle “1” undiluted on “Fresh Mode” to establish the upper range of the linearity curve. Record the results on the enclosed data sheet.
- **Second level:** Place into empty bottle “2”, 0.8 mL from bottle “1” and 0.2 mL from bottle “D” (the diluent). This will create an 80/20 dilution. Run and record results on the enclosed data sheet.
- **Third level:** Place into empty bottle “3”, 0.6 mL from bottle “1” and 0.4 mL from bottle “D” (the diluent). This will create a 60/40 dilution. Run and record results on the enclosed data sheet.
- **Fourth level:** Place into empty bottle “4”, 0.4 mL from bottle “1” and 0.6 mL from bottle “D” (the diluent). This will create a 40/60 dilution. Run and record results on the enclosed data sheet.
- **Fifth level:** Place into empty bottle “5”, 0.2 mL from bottle “1” and 0.8 mL from bottle “D” (the diluent). This will create a 20/80 dilution. Run and record results on the enclosed data sheet.
- **Sixth level:** Run bottle “D” (the diluent) as is for a 0/100 dilution. Run and record results on the enclosed data sheet.
- Congratulations! You have finished the Linearity challenge – make sure you record all your results!

STEP 2: QWIKCHECK GOLD PRECISION & LOWER LIMIT DETECTION PROTOCOL

NOTE: Before opening each sample bottle, swirl the beads 10~15 times in each direction for proper mixing.

NOTE: Run PRECISION & LOWER LIMIT DETECTION samples on the QwikCheck GOLD **CONTROL** mode.

- QwikCheck GOLD PRECISION & LOWER LIMIT DETECTION Validation Sample Testing and Reporting:
 - From the MAIN MENU select: RUN CONTROLS.
 - Select **LEVEL #1** for the bottles marked #1 through #4 and **NEGATIVE CONTROL** for bottle #5 and press ENTER.
 - Follow the onscreen testing instructions.
 - Repeat each sample 5 times back-to-back in the same Testing Capillary.
 - Record your results.

NOTE: To run this protocol, use the five (5) **Green lettered “Precision/Accuracy”** bottles marked “1” through “5”. Run 5 replicates for each sample using the same testing capillary.

NOTE: Save the remaining sample after use as these bottles will be used for the Accuracy comparison as well.

One of the samples (#5) is a low end control designed to test the lower limit detection (LLD) of the QwikCheck GOLD (0.0 M/mL). In addition to the zero level, there are samples above and below normal/abnormal cutoffs (based on WHO 5th references).

- **First level:** Run 5 replicates of sample “1” on “Control Mode **LEVEL 1**”. Record the results on the enclosed data sheet.
- **Second level:** Run 5 replicates of sample “2” on “Control Mode **LEVEL 1**”. Record results on the enclosed data sheet.
- **Third level:** Run 5 replicates of sample “3” on “Control Mode **LEVEL 1**”. Record results on the included data sheet.
- **Forth level:** Run 5 replicates of sample “4” on “Control Mode **LEVEL 1**”. Record results on the included data sheet.
- **Fifth level:** Run 5 replicates of sample “5” on “Control Mode **NEGATIVE CONTROL**”. Record results on the included data sheet.

- Save all unused samples for the Accuracy Comparison!

Suggested Live Sample Precision Protocol (Optional):

To establish precision for motility based parameters, 2 fresh human semen samples with motility >30% can be run in replicates of 5 following the steps below. This additional validation must be run on the QwikCheck GOLD **FRESH** mode.

NOTE: Use high quality liquefied human sperm samples and test within 30 minutes of collection to ensure replicate stability. Samples should be thoroughly mixed by aspirating in and out 10 times with a medium bore transfer pipette.

NOTE: HUMAN Precision samples must be run on the system's **FRESH** mode.

- From the MAIN MENU select TEST NEW PATIENT.
- Enter only the mandatory information below (you may skip all other fields):
 - Patient ID: Use a Patient ID # of your choosing.
 - Sample Type: FRESH.
 - WBC CONC: < 1 M/ml or > 1 M/ml as applicable.
- Continue pressing ENTER to advance to the Auto-Calibration screen.
- Wait for Auto-Calibration to complete without touching the system and run the testing capillary.
- Record Results.
- Test each sample 5 times back-to-back in the **Same** Testing Capillary by selecting the option "RE-TEST SAME PATIENT" from the MAIN MENU.
- **NOTE:** Time is of the essence – Run the samples back-to-back as quickly as possible.
- Record All Results.

STEP 3: QWIKCHECK GOLD CONCENTRATION ACCURACY

NOTE: To run this protocol use the five (5) **Green lettered "Precision/Accuracy"** bottles marked "1" through "5" from the Precision portion of this Validation.

NOTE: Your results from the **Precision & Lower Limit Detection** portion of this Validation will serve as the AUTOMATED RESULTS in this Accuracy comparison.

NOTE: The **Linearity** samples **CANNOT** be compared to Manual Analysis.

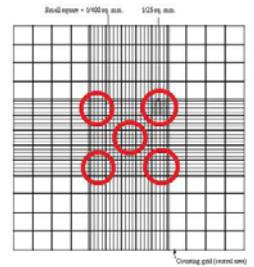
NOTE: Follow the steps below to manually count all 5 levels under the microscope using hemocytometer (Improved Neubauer) or fixed coverslip.

Neubauer Counting Chamber (100-micron depth, chamber requires sample dilution):

Follow the manufacturer's instructions for use of the Neubauer hemocytometer and the WHO Manual Guidelines for assessing sperm concentration (WHO Manual, Section 2.7, 5th Edition):

1. Dilute the beads with distilled water per WHO recommendation (WHO Manual, Section 2.8.1, 5th Edition). Do not use undiluted samples. Minimal dilution is 1:2 (1+1).
2. Thoroughly mix the material before aspirating a sample so that the beads are evenly distributed.

3. Secure the coverslip on the counting chamber.
4. Transfer 10 μ l of the diluted sample to each of the counting areas of the hemocytometer.
5. Incubate the hemocytometer for 5 minutes in a humid chamber (the beads will sediment).
6. Count the beads at a magnification of x200 – x400 using five 1/25 sq. mm areas in the center large square as shown in the grid.
7. Run duplicate counts of at least 200 beads for each sample, using new aliquot, and counting 5 squares each time. Refer to table 2.4 of the WHO Manual, 5th Edition to determine if the sum and difference of the two counts for each level are acceptable. Run new samples if not.
8. Calculate the results according to the WHO scheme (WHO Manual, Section 2.8.4, 5th Edition).
9. Repeat steps 1-8 for all remaining samples.
10. Record both replicate data of each level in the assessment form (**Appendix**).



Makler Counting Chamber (10-micron depth, chamber requires no dilution):

Follow the manufacturer's instructions for use of the Makler counting chamber in the section labeled: "Sperm Count".

1. Thoroughly mix the material before aspirating a sample so that the beads are evenly distributed. Sample dilution is not required for the Makler counting chamber.
2. Insure that the glass surfaces are clean and free of dust.
3. Place a small drop of sample in the center of the lower disc.
4. Place the cover glass on the four tips to evenly disperse a 10-micron thick bead sample over the lower disc.
5. Set microscope magnification to x 200. Locate the grid in the center of the view area.
6. Run duplicate counts of at least 200 beads. Refer to table 2.4 of the WHO Manual, 5th Edition to determine if the sum and difference of the two counts for each level are acceptable. Run new samples if not.
7. Add the duplicate counts together and divide by 2 to get an average of the two counts.
8. The number of beads in a strip of 10 squares represents concentration in millions/ml. Therefore, if 5 strips were counted divide the sum by 5.
9. Repeat steps 1-8 for all remaining samples.
10. Record both replicate data of each level in the assessment form (**Appendix**).

Counting Beads Using Microscope and Fixed Coverslip Slides (20-micron depth / no dilution, microscopic field of view must be established):

Follow the manufacturer's instructions for use of the fixed coverslip type counting chamber.

1. Fixed coverslip counting chambers may not have a scaled counting area therefore the microscopic field of view must be determined in order to achieve an accurate count. Set the microscope to either X200 or X400 magnification. To determine the field of view, use a graded ocular or a scaled commercial slide under the microscope. The formula for converting # beads/field of view into concentration in millions per ml is: $C = N/F$ (C= concentration in M/ml; N= # beads counted per field of view; F= conversion factor). If the conversion factor is not specified by the manufacturer, it can be established by multiplying the field of view by the chamber depth times 1000. (Example: If the field of view area is 0.159 mm² and the chamber depth is 20 microns the conversion factor is: 0.159 (mm²) X 0.02 (mm) X 1000 (to convert to M/ml) = 3.18. If, in the microscope's field of view 138 beads were counted, the bead concentration will be 138/3.18 = 43.4 M/ml).
2. Thoroughly mix the material before aspirating a sample so that the beads are evenly distributed. Sample dilution is usually not required for fixed coverslip chambers.
3. Load the slide with 5 μ l of sample in each of the two chambers from the two sides of the fixed coverslip slide.
4. Set the magnification to x200 or x400 and position the field of view 1/3 of the distance between the chamber opening and the opposite wall.
5. Run duplicate counts of 200 cells by counting the two chambers of the slide. Refer to table 2.4 of the WHO Manual, 5th Edition to determine if the sum and difference of the two counts for each level are acceptable. Run new samples if not.
6. Add the duplicate counts together and divide by 2 to get an average of the two counts.
7. Convert the beads to M/ml according to the formula described in #1 above.

8. Repeat steps 2-7 for all remaining samples.
9. Record both replicate data of each level in the assessment form (**Appendix**).

Table 2.4 Acceptable differences between two replicate counts for a given sum

Sum	Acceptable Difference*	Sum	Acceptable Difference*
144-156	24	329-346	36
157-169	25	347-366	37
170-182	26	367-385	38
183-196	27	386-406	39
197-211	28	407-426	40
212-226	29	427-448	41
227-242	30	449-470	42
243-258	31	471-492	43
259-274	32	493-515	44
275-292	33	516-538	45
293-309	34	539-562	46
310-328	35	563-587	47

**Based on the rounded 95% confidence interval.*

STEP 4: REPORTING RESULTS

NOTE: Medical Electronic Systems requests that all data collected during the validation be delivered in electronic format in the EXCEL template provided on the CD supplied with the kit. Simply enter your results into the EXCEL spreadsheet of the template, attach the file to an email and send to: USA Customers: service@mes-llc.com Other Customers: support@mes-ltd.com.

If this is not possible, manually enter results in the forms provided in the APPENDIX section. However, the turnaround timeframe for results may be delayed. If electronic reporting is not possible, fax a copy of the enclosed customer report to Medical Electronic Systems via FAX #: USA Customers: 866-557-9064. All other customers: 972-4-637-3984.

The QwikCheck GOLD Precision, Linearity, and Accuracy validation results will be compiled and sent to you within 10 business days. Please contact MES at any time during the validation process by calling our service line @ USA: 866-557-9064. All other customers: 972-4-637-3981 EXT 103 or by writing an e-mail to: USA: service@mes-llc.com or All other customers: support@mes-ltd.com.

QwikCheck™ Beads Precision and Linearity Kit Instructions

SQA-V GOLD

OVERVIEW

The QwikCheck™ Beads Precision and Linearity Kit is designed as a semi-annual validation tool for QwikCheck GOLD, SQA-V and SQA-VISION sperm quality analyzers. It can be used to validate Linearity, Precision and Concentration Accuracy per the 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) and Validation regulations for laboratories performing moderate and high complexity testing. These regulations also contain specific method validation requirements for modified moderate and high complexity tests and tests developed in-house. The approach in method validation is to perform a series of experiments designed to estimate certain types of analytical errors: A linearity trial to determine the system response to sequential dilutions covering the device's reportable range; a replication trial to estimate imprecision or random error; a detection limit to characterize analytical sensitivity; a comparison of the automated to the manual method vs. pre-assayed target ranges to estimate inaccuracies or systematic errors for each method and assess the agreement between two. After successfully completing this validation, the system users will have satisfied the semi-annual system validation recommendation from Medical Electronic Systems. Depending on State and Local requirements, further validation may be required.

The pre-assayed target ranges of the samples in this kit will not be known to the user (blind study), but will be applied for both automated and manual assessments to demonstrate agreement between the methods. The instructions are broken into three documents: for **QwikCheck GOLD**, **SQA-V** and **SQA-VISION**.

The tests contained in this kit should all be run following best laboratory practices – samples should be thoroughly swirled for mixing prior to capillary aspiration, quality calibrated pipettes should be used, and all manual analysis techniques should follow WHO recommended protocols.

SQA-V SYSTEM SET-UP and GENERAL INFORMATION

- After powering on the SQA and V-Sperm, print a copy of the Service Data report.
- Check the report to verify the Reference 2 (REF 2) value is within the recommended range of 2,800 to 3,200 mV.
- Set-up the SQA-V and V-Sperm default settings as described below:

From the SQA V MAIN MENU select SERVICE then SERVICE DATA.

In V-SPERM select SET-UP then SQA-V then SQA-V DEFAULTS. The following settings should be selected for the QwikCheck™ Beads Precision and Linearity Kit (you may return them to your specific settings afterwards):

- ✓ Conc. Standard: 2.
- ✓ LES:1US.
- ✓ Printing Options: Automatically print all test results.
- ✓ Printing Options: Automatically print Self-Test Report on Start Up.
- ✓ Controls: Latex Beads.
- ✓ LEVEL 1 Target and Range = 100 +/- 99.9.
- ✓ LEVEL 2 Target and Range = 100 +/- 99.9.
- ✓ NEGATIVE CONTROL = 0.0.

STEP 1: SQA-V LINEARITY & REPORTABLE RANGE PROTOCOL

NOTE: Before opening each sample bottle, swirl the beads 10~15 times in each direction for proper mixing.

NOTE: Run LINEARITY & REPORTABLE RANGE samples on the SQA's **TEST PATIENT** mode.

- SQA-V LINEARITY& REPORTABLE RANGE Sample Testing and Reporting:
 - From the MAIN MENU select: TEST NEW PATIENT.
 - Enter only the mandatory information below (you may skip all other fields):
 - Patient ID: Use the sample # on the top of each testing bottle.
 - Sample Type: FRESH.
 - WBC CONC: < 1 M/ml.
 - Continue pressing ENTER to advance to the Auto-Calibration screen.
 - Wait for Auto-Calibration to complete without touching the system and then run the testing capillary.
 - Record your results.

NOTE: To run this protocol use the six (6) **Blue Lettered "Linearity"** bottles marked "1" through "5" and the bottle marked "D". Using a quality calibrated pipette, make serial dilutions from bottle "1" by following the instructions below. Record results on the data sheet provided in the kit or in the Validation Data Entry form (Excel). The message: "Low Quality Sample testing will take 2 more minutes" may be displayed during some tests. This is normal and occurs because there is no motility present.

- **First level:** Run bottle "1" undiluted on "Fresh Mode" to establish the upper range of the linearity curve. Record the results on the enclosed data sheet.
- **Second level:** Place into empty bottle "2", 0.8 mL from bottle "1" and 0.2 mL from bottle "D" (the diluent). This will create an 80/20 dilution. Run and record results on the enclosed data sheet.

- **Third level:** Place into empty bottle “3”, 0.6 mL from bottle “1” and 0.4 mL from bottle “D” (the diluent). This will create a 60/40 dilution. Run and record results on the enclosed data sheet.
- **Fourth level:** Place into empty bottle “4”, 0.4 mL from bottle “1” and 0.6 mL from bottle “D” (the diluent). This will create a 40/60 dilution. Run and record results on the enclosed data sheet.
- **Fifth level:** Place into empty bottle “5”, 0.2 mL from bottle “1” and 0.8 mL from bottle “D” (the diluent). This will create a 20/80 dilution. Run and record results on the enclosed data sheet.
- **Sixth level:** Run bottle “D” (the diluent) as is for a 0/100 dilution. Run and record results on the enclosed data sheet.
- Congratulations! You have finished the Linearity challenge – make sure you record all your results!

STEP 2: SQA-V PRECISION & LOWER LIMIT DETECTION PROTOCOL

NOTE: Before opening each sample bottle, swirl the beads 10~15 times in each direction for proper mixing.

NOTE: Run PRECISION & LOWER LIMIT DETECTION samples on the SQA **CONTROL** mode.

- SQA-V PRECISION & LOWER LIMIT DETECTION Validation Sample Testing and Reporting:
 - From the SQA MAIN MENU select: RUN CONTROLS.
 - Select **LEVEL #1** for the bottles marked #1 through #4 and **NEGATIVE CONTROL** for bottle #5 and press ENTER.
 - Follow the onscreen testing instructions.
 - Repeat each sample 5 times back-to-back in the same Testing Capillary.
 - Record your results.

NOTE: To run this protocol, use the five (5) **Green lettered “Precision/Accuracy”** bottles marked “1” through “5”. Run 5 replicates for each sample using the same testing capillary.

NOTE: Save the remaining sample after use as these bottles will be used for the Accuracy comparison as well.

One of the samples (#5) is a low end control designed to test the lower limit detection (LLD) of the SQA (0.0 M/mL). In addition to the zero level, there are samples above and below normal/abnormal cutoffs (based on WHO 5th references).

- **First level:** Run 5 replicates of sample “1” on “Control Mode **LEVEL 1**”. Record the results on the enclosed data sheet.
- **Second level:** Run 5 replicates of sample “2” on “Control Mode **LEVEL 1**”. Record results on the enclosed data sheet.
- **Third level:** Run 5 replicates of sample “3” on “Control Mode **LEVEL 1**”. Record results on the included data sheet.
- **Forth level:** Run 5 replicates of sample “4” on “Control Mode **LEVEL 1**”. Record results on the included data sheet.
- **Fifth level:** Run 5 replicates of sample “5” on “Control Mode **NEGATIVE CONTROL**”. Record results on the included data sheet.
- Save all unused samples for the Accuracy Comparison!

Suggested Live Sample Precision Protocol (Optional):

To establish precision for motility based parameters, 2 fresh human semen samples with motility >30% can be run in replicates of 5 following the steps below. This additional validation must be run on the SQA's **FRESH** mode.

NOTE: Use high quality liquefied human sperm samples and test within 30 minutes of collection to ensure replicate stability. Samples should be thoroughly mixed by aspirating in and out 10 times with a medium bore transfer pipette.

NOTE: HUMAN Precision samples must be run on the system's **FRESH** mode.

- From the MAIN MENU select TEST NEW PATIENT.
- Enter only the mandatory information below (you may skip all other fields):
 - Patient ID: Use a Patient ID # of your choosing.
 - Sample Type: FRESH.
 - WBC CONC: < 1 M/ml or > 1 M/ml as applicable.
- Continue pressing ENTER to advance to the Auto-Calibration screen.
- Wait for Auto-Calibration to complete without touching the system and run the testing capillary.
- Record Results.
- Test each sample 5 times back-to-back in the **Same** Testing Capillary by selecting the option "RE-TEST SAME SAMPLE" from the MAIN MENU.
- **NOTE:** Time is of the essence – Run the samples back-to-back as quickly as possible.
- Record All Results.

STEP 3: SQA-V CONCENTRATION ACCURACY

NOTE: To run this protocol use the five (5) **Green lettered "Precision/Accuracy"** bottles marked "1" through "5" from the Precision portion of this Validation.

NOTE: Your results from the **Precision & Lower Limit Detection** portion of this Validation will serve as the AUTOMATED RESULTS in this Accuracy comparison.

NOTE: The **Linearity** samples **CANNOT** be compared to Manual Analysis.

NOTE: Follow the steps below to manually count all 5 levels under the microscope or in the SQA Visualization Chamber. The options are: hemocytometer (Improved Neubauer), fixed coverslip side using the SQA-V visualization or microscope.

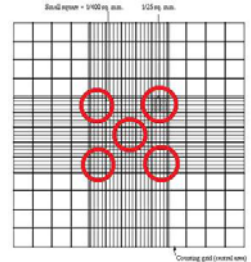
Counting Beads Using the SQA-V Visualization System and Fixed Coverslip Slides:

1. Refer to the SQA-V User Guide for instructions how to use the SQA visualization system.
2. To COUNT beads: Press **ZOOM-OUT** to the maximum magnification.
3. Prepare a slide: Mix sample thoroughly and load ~5 µl in SQA-VISION fixed coverslip slide.
4. Insert the slide into the slide adaptor and then into the visualization chamber. Adjust **CONTRAST, COLOR, BRIGHTNESS** and **FOCUS** controls for optimal image quality as necessary.
5. Open V-Sperm and click on the Real Time Video button.
6. Turn slide adaptor knob full counterclockwise and FREEZE the image.
7. Count 10 fields of view using a lab counter. Turn the slide adaptor knob to view multiple fields.
8. Repeat steps 3-8 four more times for each of the 5 levels.
9. Record the duplicate results of each level in the assessment form (**Appendix**).

Neubauer Counting Chamber (100-micron depth, chamber requires sample dilution):

Follow the manufacturer's instructions for use of the Neubauer hemocytometer and the WHO Manual Guidelines for assessing sperm concentration (WHO Manual, Section 2.7, 5th Edition):

1. Dilute the beads with distilled water per WHO recommendation (WHO Manual, Section 2.8.1, 5th Edition). Do not use undiluted samples. Minimal dilution is 1:2 (1+1).
2. Thoroughly mix the material before aspirating a sample so that the beads are evenly distributed.
3. Secure the coverslip on the counting chamber.
4. Transfer 10 µl of the diluted sample to each of the counting areas of the hemocytometer.
5. Incubate the hemocytometer for 5 minutes in a humid chamber (the beads will sediment).
6. Count the beads at a magnification of x200 – x400 using five 1/25 sq. mm areas in the center large square as shown in the grid.
7. Run duplicate counts of at least 200 beads for each sample, using new aliquot, and counting 5 squares each time. Refer to table 2.4 of the WHO Manual, 5th Edition to determine if the sum and difference of the two counts for each level are acceptable. Run new samples if not.
8. Calculate the results according to the WHO scheme (WHO Manual, Section 2.8.4, 5th Edition).
9. Repeat steps 1-8 for all remaining samples.
10. Record both replicate data of each level in the assessment form (**Appendix**).



Makler Counting Chamber (10-micron depth, chamber requires no dilution):

Follow the manufacturer's instructions for use of the Makler counting chamber in the section labeled: "Sperm Count".

11. Thoroughly mix the material before aspirating a sample so that the beads are evenly distributed. Sample dilution is not required for the Makler counting chamber.
12. Insure that the glass surfaces are clean and free of dust.
13. Place a small drop of sample in the center of the lower disc.
14. Place the cover glass on the four tips to evenly disperse a 10-micron thick bead sample over the lower disc.
15. Set microscope magnification to x 200. Locate the grid in the center of the view area.
16. Run duplicate counts of at least 200 beads. Refer to table 2.4 of the WHO Manual, 5th Edition to determine if the sum and difference of the two counts for each level are acceptable. Run new samples if not.
17. Add the duplicate counts together and divide by 2 to get an average of the two counts.
18. The number of beads in a strip of 10 squares represents concentration in millions/ml. Therefore, if 5 strips were counted divide the sum by 5.
19. Repeat steps 1-8 for all remaining samples.
20. Record both replicate data of each level in the assessment form (**Appendix**).

Counting Beads Using Microscope and Fixed Coverslip Slides (20-micron depth / no dilution, microscopic field of view must be established):

Follow the manufacturer's instructions for use of the fixed coverslip type counting chamber.

1. Fixed coverslip counting chambers may not have a scaled counting area therefore the microscopic field of view must be determined in order to achieve an accurate count. Set the microscope to either X200 or X400 magnification. To determine the field of view, use a graded ocular or a scaled commercial slide under the microscope. The formula for converting # beads/field of view into concentration in millions per ml is: $C = N/F$ (C = concentration in M/ml; N = # beads counted per field of view; F = conversion factor). If the conversion factor is not specified by the manufacturer, it can be established by multiplying the field of view by the chamber depth times 1000. (Example: If the field of view area is 0.159 mm² and the chamber depth is 20 microns the conversion factor is: 0.159 (mm²) X 0.02 (mm) X 1000 (to convert to M/ml) = 3.18. If, in the microscope's field of view 138 beads were counted, the bead concentration will be 138/3.18 = 43.4 M/ml)
2. Thoroughly mix the material before aspirating a sample so that the beads are evenly distributed. Sample dilution is usually not required for fixed coverslip chambers.

3. Load the slide with 5 µl of sample in each of the two chambers from the two sides of the Vision fixed coverslip slide.
4. Set the magnification to x200 or x400 and position the field of view 1/3 of the distance between the chamber opening and the opposite wall.
5. Run duplicate counts of 200 cells by counting the two chambers of the slide. Refer to table 2.4 of the WHO Manual, 5th Edition to determine if the sum and difference of the two counts for each level are acceptable. Run new samples if not.
6. Add the duplicate counts together and divide by 2 to get an average of the two counts.
7. Convert the beads to M/ml according to the formula described in #1 above.
8. Repeat steps 2-8 for all remaining samples.
9. Record both replicate data of each level in the assessment form (**Appendix**).

Table 2.4 Acceptable differences between two replicate counts for a given sum

Sum	Acceptable Difference*	Sum	Acceptable Difference*
144–156	24	329–346	36
157–169	25	347–366	37
170–182	26	367–385	38
183–196	27	386–406	39
197–211	28	407–426	40
212–226	29	427–448	41
227–242	30	449–470	42
243–258	31	471–492	43
259–274	32	493–515	44
275–292	33	516–538	45
293–309	34	539–562	46
310–328	35	563–587	47

*Based on the rounded 95% confidence interval.

STEP 4: REPORTING RESULTS

NOTE: Medical Electronic Systems requests that all data collected during the validation be delivered in electronic format in the EXCEL template provided on the CD supplied with the kit. Simply enter your results into the EXCEL spreadsheet of the template, attach the file to an email and send to: USA Customers: service@mes-llc.com Other Customers: support@mes-ltd.com.

If this is not possible, manually enter results in the forms provided in the APPENDIX section. However, the turnaround timeframe for results may be delayed. If electronic reporting is not possible, fax a copy of the enclosed customer report to Medical Electronic Systems via FAX #: USA Customers: 866-557-9064. All other customers: 972-4-637-3984.

The SQA Precision, Linearity, and Accuracy validation results will be compiled and sent to you within 10 business days. Please contact MES at any time during the validation process by calling our service line @ USA: 866-557-9064. All other customers: 972-4-637-3981 EXT 103 or by writing an e-mail to: USA: service@mes-llc.com or All other customers: support@mes-ltd.com.

QwikCheck™ Beads Precision and Linearity Kit Instructions

SQA-Vision

OVERVIEW

The QwikCheck™ Beads Precision and Linearity Kit is designed as a semi-annual validation tool for QwikCheck GOLD, SQA-V and SQA-VISION sperm quality analyzers. It can be used to validate Linearity, Precision and Concentration Accuracy per the 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) and Validation regulations for laboratories performing moderate and high complexity testing. These regulations also contain specific method validation requirements for modified moderate and high complexity tests and tests developed in-house. The approach in method validation is to perform a series of experiments designed to estimate certain types of analytical errors: A linearity trial to determine the system response to sequential dilutions covering the device's reportable range; a replication trial to estimate imprecision or random error; a detection limit to characterize analytical sensitivity; a comparison of the automated to the manual method vs. pre-assayed target ranges to estimate inaccuracies or systematic errors for each method and assess the agreement between two. After successfully completing this validation, the system users will have satisfied the semi-annual system validation recommendation from Medical Electronic Systems. Depending on State and Local requirements, further validation may be required.

The pre-assayed target ranges of the samples in this kit will not be known to the user (blind study), but will be applied for both automated and manual assessments to demonstrate agreement between the methods. The instructions are broken into three documents: for **QwikCheck GOLD**, **SQA-V** and **SQA-VISION**.

The tests contained in this kit should all be run following best laboratory practices – samples should be thoroughly swirled for mixing prior to capillary aspiration, quality calibrated pipettes should be used, and all manual analysis techniques should follow WHO recommended protocols.

SQA-Vision SYSTEM SET-UP and GENERAL INFORMATION

- After powering on the SQA-VISION PC and SQA unit print a copy of the Service Data report.
- Check the report to verify the Reference 2 (REF 2) value is within the recommended range of 2,800 to 3,200 mV.
- Set-up the SQA-VISION default settings as described below:

From the MAIN MENU select SETTINGS then CONTROLS and enter the following values manually:

- ✓ LEVEL 1 Target and Range = 100 +/- 100.
- ✓ LEVEL 2 Target and Range = 100 +/- 100.
- ✓ NEGATIVE CONTROL = 0.0.

From the MAIN MENU select SETTINGS then TEST PATIENT. The following settings should be selected for the QwikCheck™ Beads Precision and Linearity Kit (you may return them to your specific settings afterwards):

- ✓ Conc. Standard: 2.
- ✓ LES: 1US.
- ✓ Debris Scanning: NONE.
- ✓ Low Quality Counter: NOT SELECTED.

Validation Kit test results will be saved separately in the PATIENT and CONTROLS archive.

STEP 1: SQA-VISION LINEARITY & REPORTABLE RANGE PROTOCOL

NOTE: Before opening each sample bottle, swirl the beads 10~15 times in each direction for proper mixing.

NOTE: Run LINEARITY & REPORTABLE RANGE samples on the VISION's **TEST PATIENT** mode.

- SQA-V LINEARITY & REPORTABLE RANGE Sample Testing and Reporting:
 - From the MAIN MENU select: TEST NEW PATIENT then FRESH.
 - Enter only the mandatory information below (you may skip all other fields):
 - Patient ID: Use the sample # on the top of each testing bottle.
 - Sample Volume: 1.0 ml.
 - WBC CONC: < 1 M/ml.
 - Choose TEST NOW to advance to the Auto-Calibration screen.
 - Wait for Auto-Calibration to complete without touching the system and then run the testing capillary.
 - Record your results.

NOTE: To run this protocol use the six (6) **Blue Lettered "Linearity"** bottles marked "1" through "5" and the bottle marked "D". Using a quality calibrated pipette, make serial dilutions from bottle "1" by following the instructions below. Record results on the data sheet provided in the kit or in the Validation Data Entry form (Excel).

- **First level:** Run bottle "1" undiluted on "Fresh Mode" to establish the upper range of the linearity curve. Record the results on the enclosed data sheet.
- **Second level:** Place into empty bottle "2", 0.8 mL from bottle "1" and 0.2 mL from bottle "D" (the diluent). This will create an 80/20 dilution. Run and record results on the enclosed data sheet.
- **Third level:** Place into empty bottle "3", 0.6 mL from bottle "1" and 0.4 mL from bottle "D" (the diluent). This will create a 60/40 dilution. Run and record results on the enclosed data sheet.

- **Fourth level:** Place into empty bottle “4”, 0.4 mL from bottle “1” and 0.6 mL from bottle “D” (the diluent). This will create a 40/60 dilution. Run and record results on the enclosed data sheet.
- **Fifth level:** Place into empty bottle “5”, 0.2 mL from bottle “1” and 0.8 mL from bottle “D” (the diluent). This will create a 20/80 dilution. Run and record results on the enclosed data sheet.
- **Sixth level:** Run bottle “D” (the diluent) as is for a 0/100 dilution. Run and record results on the enclosed data sheet.
- Congratulations! You have finished your Linearity challenge – make sure you record all your results!

STEP 2: SQA-VISION PRECISION - REPLICATION & LOWER LIMIT DETECTION

NOTE: Before opening each sample bottle, swirl the beads 10~15 times in each direction for proper mixing.

NOTE: Run PRECISION & LOWER LIMIT DETECTION samples on the VISION CONTROL mode.

- SQA-V PRECISION & LOWER LIMIT DETECTION Validation Sample Testing and Reporting:
 - From the SQA MAIN MENU select QC/PROFICIENCY then LATEX BEADS.
 - Select **LEVEL #1** for the bottles marked #1 through #4 and **NEGATIVE CONTROL** for bottle #5 and press TEST NOW.
 - Follow the onscreen testing instructions.
 - Repeat each sample 5 times back-to-back in the same Testing Capillary.
 - Record your results.

NOTE: To run this protocol use the five (5) **Green lettered “Precision/Accuracy”** bottles marked “1” through “5”. Run 5 replicates for each sample using the same testing capillary.

NOTE: Save the remaining sample after use as these bottles will be used for the Accuracy comparison as well.

One of the samples is a low end control designed to test the lower limit detection (LLD) of the SQA (0.0 M/mL). In addition to the zero level, there are samples above and below normal/abnormal cutoffs (based on WHO 5th references).

- **First level:** Run 5 replicates of sample “1” on “QC/Proficiency > Latex Beads **Level 1**” Mode. Record the results on the data sheet.
- **Second level:** Run 5 replicates of sample “2” on “QC/Proficiency > Latex Beads **Level 1**” Mode. Record the results on the data sheet.
- **Third level:** Run 5 replicates of sample “3” on “QC/Proficiency > Latex Beads **Level 1**” Mode. Record the results on the data sheet.
- **Forth level:** Run 5 replicates of sample “4” on “QC/Proficiency > Latex Beads **Level 1**” Mode. Record the results on the data sheet.
- **Fifth level:** Run 5 replicates of sample “5” on “QC/Proficiency” Mode > Latex Beads **Negative Control**” Mode. Record the results on the data sheet.
- Save all unused samples for the Accuracy Comparison!

Suggested Live Sample Precision Protocol (Optional):

To establish precision for motility based parameters, 2 fresh human semen samples with motility >30% can be run in replicates of 5 following the steps below. This additional validation must be run on the SQA **FRESH** mode.

NOTE: Use high quality fresh human sperm samples and test within 30 minutes of collection to ensure replicate stability. Samples should be thoroughly mixed by aspirating in and out 10 times with a medium bore transfer pipette.

NOTE: HUMAN Precision samples must be run on the system's **TEST PATIENT ->FRESH** mode.

- From the MAIN MENU select **TEST NEW PATIENT** then choose **FRESH**.
- Enter only the mandatory information below (you may skip all other fields):
 - Patient ID: Use the sample # on the top of each testing bottle.
 - Sample Volume: 1.0 ml.
 - WBC CONC: < 1 M/ml or > 1 M/ml as applicable.
- Press TEST NOW to advance to the Auto-Calibration screen.
- Wait for Auto-Calibration to complete without touching the system and run the testing capillary.
- Record Results.
- Test each sample 5 times back-to-back in the **Same** Testing Capillary by selecting the option "RE-TEST SAME SAMPLE" from the Results Screen.
- **NOTE:** Time is of the essence – Run the samples back-to-back as quickly as possible.
- Record All Results!

STEP 3: SQA-VISION CONCENTRATION ACCURACY

NOTE: To run this protocol use the five (5) **Green lettered "Precision/Accuracy"** bottles marked "1" through "5" from the Precision portion of this Validation.

NOTE: Your results from the **Precision & Lower Limit Detection** portion of this Validation will serve as the AUTOMATED RESULTS in this Accuracy comparison.

NOTE: The **Linearity** samples **CANNOT** be compared to Manual Analysis.

NOTE: Follow the steps below to manually count all 5 levels under the microscope or in the SQA Visualization Chamber. The options are: hemocytometer and fixed coverslip side using visualization chamber or microscope.

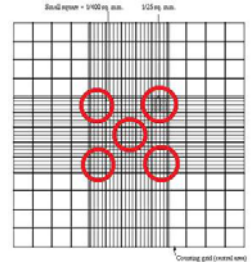
Counting Beads Using the SQA-Vision Visualization System and Fixed Coverslip Slides:

1. Refer to the SQA-Vision User Guide (Appendix 3) for instructions how to use a slide in the SQA visualization system.
2. Select VISUALIZATION from the MAIN MENU to open the video screen. Click the **CAPTURE** button in the upper right-hand corner.
3. To COUNT beads: Press **ZOOM-IN** to the maximum magnification.
4. Prepare a slide: Mix sample thoroughly and load ~5 µl in SQA-VISION fixed coverslip slide.
5. Insert the slide into the Visualization Field of View Stage.
6. Adjust the FOCUS knob to make the image clear.
7. Turn Field of View knob full counterclockwise.
8. Count 10 fields of view using lab counter. Turn the Field of View knob to view multiple fields.
9. Repeat steps 4-8 four more times for each of the 5 levels.
10. Record the duplicate data of each level in the assessment form (**Appendix**).

Neubauer Counting Chamber (100-micron depth, chamber requires sample dilution):

Follow the manufacturer's instructions for use of the Neubauer hemocytometer and the WHO Manual Guidelines for assessing sperm concentration (WHO Manual, Section 2.7, 5th Edition):

1. Dilute the beads with distilled water per WHO recommendation (WHO Manual, Section 2.8.1, 5th Edition). Do not use undiluted samples. Minimal dilution is 1:2 (1+1).
2. Thoroughly mix the material before aspirating a sample so that the beads are evenly distributed.
3. Secure the coverslip on the counting chamber.
4. Transfer 10 μ l of the diluted sample to each of the counting areas of the hemocytometer.
5. Incubate the hemocytometer for 5 minutes in a humid chamber (the beads will sediment).
6. Count the beads at a magnification of x200 – x400 using five 1/25 sq. mm areas in the center large square as shown in the grid.
7. Run duplicate counts of at least 200 beads for each sample, using new aliquot, and counting 5 squares each time. Refer to table 2.4 of the WHO Manual, 5th Edition to determine if the sum and difference of the two counts for each level are acceptable. Run new samples if not.
8. Calculate the results according to the WHO scheme (WHO Manual, Section 2.8.4, 5th Edition).
9. Repeat steps 2-8 four more times to achieve 5 replicates for each sample.
10. Record both replicate data of each level in the assessment form (**Appendix**).



Makler Counting Chamber (10-micron depth, chamber requires no dilution):

Follow the manufacturer's instructions for use of the Makler counting chamber in the section labeled: "Sperm Count".

1. Thoroughly mix the material before aspirating a sample so that the beads are evenly distributed. Sample dilution is not required for the Makler counting chamber.
2. Insure that the glass surfaces are clean and free of dust.
3. Place a small drop of sample in the center of the lower disc.
4. Place the cover glass on the four tips to evenly disperse a 10-micron thick bead sample over the lower disc.
5. Set microscope magnification to x 200. Locate the grid in the center of the view area.
6. Run duplicate counts of at least 200 beads. Refer to table 2.4 of the WHO Manual, 5th Edition to determine if the sum and difference of the two counts for each level are acceptable. Run new samples if not.
7. Add the duplicate counts together and divide by 2 to get an average of the two counts.
8. The number of beads in a strip of 10 squares represents concentration in millions/ml. Therefore, if 5 strips were counted divide the sum by 5.
9. Repeat steps 1-8 for all remaining samples.
10. Record both replicate data of each level in the assessment form (**Appendix**).

Counting Beads Using Microscope and Fixed Coverslip Slides (20-micron depth / no dilution, microscopic field of view must be established):

Follow the manufacturer's instructions for use of the fixed coverslip type counting chamber.

1. Fixed coverslip counting chambers may not have a scaled counting area therefore the microscopic field of view must be determined in order to achieve inaccurate count. Set the microscope to either X200 or X400 magnification. To determine the field of view, use a graded ocular or a scaled commercial slide under the microscope. The formula for converting # beads/field of view is: $C = N/F$ (C= concentration in M/ml; N= # beads counted per field of view; F= conversion factor). If the conversion factor is not specified by the manufacturer, it can be established by multiplying the field of view by the chamber depth times 1000. (Example: If the field of view area is 0.159 mm² and the chamber depth is 20 microns the conversion factor is: 0.159 (mm²) X 0.02 (mm) X 1000 (to convert to M/ml) = 3.18. If, in the microscope's field of view 138 beads were counted, the bead concentration will be 138/3.18 = 43.4 M/ml)
2. Thoroughly mix the material before aspirating a sample so that the beads are evenly distributed. Sample dilution is usually not required for fixed coverslip chambers.

3. Load the slide with 5 µl of sample in each of the two chambers from the two sides of the fixed coverslip slide.
4. Set the magnification to x200 or x400 and position the field of view 1/3 of the distance between the chamber opening and the opposite wall.
5. Run duplicate counts of 200 cells by counting the two chambers of the slide. Refer to table 2.4 of the WHO Manual, 5th Edition to determine if the sum and difference of the two counts for each level are acceptable. Run new samples if not.
6. Add the duplicate counts together and divide by 2 to get an average of the two counts.
7. Convert the beads to M/ml according to the formula described in #1 above.
8. Repeat steps 2-7 four more times to achieve 5 replicates for each sample.
9. Record both replicate data of each level in the assessment form (**Appendix**).

Table 2.4 Acceptable differences between two replicate counts for a given sum

Sum	Acceptable Difference*	Sum	Acceptable Difference*
144–156	24	329–346	36
157–169	25	347–366	37
170–182	26	367–385	38
183–196	27	386–406	39
197–211	28	407–426	40
212–226	29	427–448	41
227–242	30	449–470	42
243–258	31	471–492	43
259–274	32	493–515	44
275–292	33	516–538	45
293–309	34	539–562	46
310–328	35	563–587	47

**Based on the rounded 95% confidence interval.*

STEP 4: REPORTING RESULTS

NOTE: Medical Electronic Systems requests that all data collected during the validation be delivered in electronic format in the EXCEL template provided on CD with the kit. Simply enter you results into the EXCEL spreadsheet of the template, attach the file to an email and send to: USA Customers: service@mes-llc.com Other Customers: support@mes-ltd.com.

If this is not possible, manually enter results in the forms provided in the APPENDIX section. However, the turnaround timeframe for results may be delayed. If electronic reporting is not possible, fax a copy of the enclosed customer report to Medical Electronic Systems via FAX #: USA Customers: 866-557-9064. All other customers: 972-4-637-3984.

The SQA Precision, Linearity, and Accuracy validation results will be compiled and sent to you within 10 business days. Please contact MES at any time during the validation process by calling our service line @ USA: 866-557-9064. All other customers: 972-4-637-3981 EXT 103 or by writing an e-mail to: USA: service@mes-llc.com or All other customers: support@mes-ltd.com.

Appendix: PRECISION & LINEARITY KIT DATA ENTRY FORM

FACILITY		KIT BATCH #	
DEVICE TYPE & SN#		DATE	

Enter data into the white fields of the tables below.

LINEARITY

Materials: QwikCheck™ Beads

Sample #	Dilution Ratio	Conc., M/ml
1	100/0	
2	80/20	
3	60/40	
4	40/60	
5	20/80	
6	0/100	

PRECISION / ACCURACY

Sample #1

Replicate #	Automated	Manual
1		
2		
3		-
4		-
5		-

Sample #2

Replicate #	Automated	Manual
1		
2		
3		-
4		-
5		-

Sample #3

Replicate #	Automated	Manual
1		
2		
3		-
4		-
5		-

Sample #4

Replicate #	Automated	Manual
1		
2		
3		-
4		-
5		-

Sample #5

Replicate #	Automated	Manual
1		
2		
3		-
4		-
5		-

Enter data into the white fields of the tables below.

OPTIONAL: PRECISION & REPLICATION: **Using Live Semen Samples**

Sample #1

Replicate #	Conc. M/mL	Motility %	Morph. %
1			
2			
3			
4			
5			

Sample #2

Replicate #	Conc. M/mL	Motility %	Morph. %
1			
2			
3			
4			
5			

Product Regulatory Information - QwikCheck™ Beads Precision and Linearity Kit



Manufacturer's Name

MES Medical Electronic Systems

Manufacturer's Address

20 Alon Hatavor Street, Zone 6, Caesarea Industrial Park, Israel
3088900



Product Part #

A-CA-01188-00



Storage and handling conditions

Store the QwikCheck™ Beads Precision and Linearity Kit @ 4-8°C (39-46°F). The expiration date assumes that QwikCheck™ Beads Precision and Linearity Kit is stored in its original containers and the bottles are tightly capped to prevent evaporation.

Number of tests included

1 round of testing for product validation



IVD Test Kit