QwikCheck™ BEADS

QUALITY CONTROL BEADS FOR AUTOMATED AND MANUAL SPERM COUNTING DEVICES

Introduction and Intended Use:

QwikCheck™ beads is an in-vitro use only external quality control material for automated and manual sperm counting systems. It has been developed as a tool to assess the accuracy and precision of the laboratory's sperm counting methods by providing a known target value and +/- range. The beads were developed for use on the SQA semen analyzer however, they are also labeled for manual proficiency testing and calibration on hemacytometers such as Neubauer counting chambers, Makler chambers and conventional fixed coverslip chambers.

QwikCheck™ beads is supplied in a kit containing known concentrations of 4-micron latex beads suspended in an aqueous solvent and negative concentration/motility control. The beads should be run according to the directions below for the type of system used in the lab. According to the CLIA '88 regulations, "…for most moderately complex tests, the general requirement is to analyze two levels of QC materials on each day of testing." It is recommended that QwikCheck beads are run on both the SQA automated and visualization systems prior to each day of semen analysis testing.

Storage Conditions:

Each kit contains two known concentrations of **QwikCheck™ beads** in two 5 ml aliquots and one 5 ml negative concentration and motility control. Store the beads @ 15-30°C (60-86°F). Open vial beads can be stored @ 15-30°C (60-86°F) for 90 days. The expiration date assumes that **QwikCheck™ beads** are stored in their original containers and tightly capped to prevent evaporation. QwikCheck™ beads are stable and show no loss of expected performance characteristics after transport/storage over a period of 72 hours at a temperature range of -20°C to +37°C (-4°F to +99°F).

Target Value and +/- Ranges

Target Values and +/- Ranges unique to the batch have established for each batch of QwikCheck beads. Each box and each control bottle is labeled with these Target Values and Ranges. In addition, the MES website: www.mes-global.com provides Batch Release Forms by batch # with details.

Warning:

Contains 0.1% Sodium Azide as a preservative. Other ingredients are not harmful due to the low concentration in the beads. For additional information, please refer to the QwikCheck-beads Safety Data Sheet # **QCB 001**

Basic Instructions for using QwikCheck™ beads

1. BEFORE initially opening new beads, turn the bottle upside down and gently shake. During shipment, some beads adhere to the top of the bottle.
2. Mix the beads in the closed container by gently rotating by hand. This will evenly distribute the beads in the suspension and insure accurate test results. Do not use a vortex except for the Neubauer chamber which requires vortexing because the beads are diluted with water.
3. Open the container and immediately withdraw a sample of the control material. Tightly close the container after withdrawing the sample.
4. Follow the detailed instructions below for the type of system/chamber used.

Instructions for running QwikCheck™ beads QC material on automated and manual sperm counting systems:

SQA Automated System:

1. Refer to the SQA User Guide "CONTROLS" section for an explanation of how to set-up the SQA to test automated Level 1/Level 2 and Negative Control QwikCheck™ beads. Follow the SQA onscreen instructions in the "Controls" section of the SQA User Guide:
2. Before opening new beads, gently rotate the closed container of beads upside down to remove any beads that may have adhered to the top liner.
3. Mix opened beads by gently rotating the close container (do not use a vortex).
4. Aspirate a sample of the beads or negative control into the SQA capillary in the same manner you would fill the capillary for a normal volume specimen. Make sure that the cuvette section of the SQA capillary is completely full of liquid and free of bubbles.
5. Insert the testing capillary into the SQA measurement chamber when prompted.
6. Use a new capillary for running each level of beads.

SQA Visualization System using a fixed coverslip slide:

1. Refer to the SQA / VISION User Guide for instructions how to use slide in the SQA visualization.
2. Gently rotate the closed container of beads by hand (do not use a vortex).
3. Pipette 5 µL of QwikCheck™ beads onto a fixed coverslip slide.
4. If air bubbles are seen, prepare a new slide to ensure accurate results.
5. Insert the slide into the slide adaptor and then into the visualization chamber of the SQA. Press Zoom-Out (SQA-V) or Zoom-In (SQA-Vision) all the way and FREEZE the image.
6. Turn the field of view knob counterclockwise to the stopper. Count the beads manually per WHO guidelines: Duplicate counts of at least 200 beads are required (turn the slide adaptor knob to view multiple fields). Divide the final number of beads counted in the multiple fields by the number of screens viewed. Each bead on the SQA / VISION screen represents 1 M/ml.
7. Refer to the WHO 5th Edition Manual, table 2.4 to determine if the duplicate counts are acceptable.

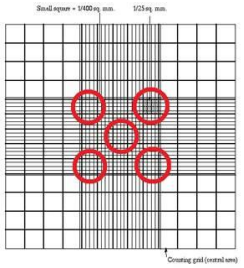
Neubauer Counting Chamber (100-micron depth, chamber requires sample dilution):

Follow the instructions below to count the QwikCheck Beads Level 1 and Level 2. These instructions follow exactly the WHO 5th guidelines for counting sperm. Refer to manufacturer's instructions for use of the Neubauer hemocytometer.

Level 1 (High):

1. Dilute 2 separate 50µl aliquots of beads with distilled water 1:4 (1 portion of beads + 3 portions of water). Vortex the beads before aspirating to assure homogeneous spread.
2. Secure the coverslip on the counting chamber.
3. Load 10µl of dilution #1 into one side of the Neubauer chamber, and dilution #2 into the second side.
4. Incubate the Neubauer chamber for 5 minutes in a humid chamber to let the beads sediment evenly.

5. Per WHO, count 200 beads in 5 squares of the central grid (as shown in grid to the right) at x200 – x400 magnification.
6. Count both sides of the Neubauer chamber and then refer to table 2.4 of the WHO Manual, 5th Edition to determine if the sum and difference of the two counts are acceptable. Run new samples if not.
7. If counts are acceptable, average the two counts and divide by 5. The results equal bead concentration in Millions /ml.
8. Compare the results to the QwikCheck™ beads target values on the box label.



Level 2 (Low):

1. Dilute 2 separate 50µl aliquots of beads with distilled water 1:2 (1 portion of beads + 1 portion of water). Vortex the beads before aspirating to assure homogeneous spread.
2. Exactly follow the Level 1 instructions steps 2-6 above.
3. If counts are acceptable, average the two counts and divide by 10. The results equal bead concentration in Millions /ml.
4. Compare the results to the QwikCheck™ beads target values on the box label.

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. Gently rotate the closed container of beads by hand (do not use a vortex). Sample dilution is not required for the Makler counting chamber.
2. Ensure that the glass surfaces are clean and free of dust.
3. Place a small drop of QwikCheck™ beads 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. Confirm that the results are acceptable by calculating the sum and difference of the two counts. 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 the results exceed the acceptable level.
7. Add the duplicate counts together and divide by 2 to get an average of the two counts.
8. The number of the beads in a strip of 10 squares represents concentration in millions/ml. Therefore, if 5 strips were counted divide the sum by 5.
9. Compare the results to the QwikCheck™ beads target values.

Fixed coverslip counting chambers (20-micron depth, requires 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 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 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 mm^2 and the chamber depth is 20 microns the conversion factor is: 0.159 (mm^2) 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. Gently rotate the closed container of beads by hand (do not use a vortex). Sample dilution is not usually required for fixed coverslip chambers.
3. Load the chamber with 5 µl of beads or negative control from the two sides of the fixed coverslip type chamber.
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 drops on opposite sides of the chamber. Confirm that the results are acceptable by calculating the sum and difference of the two counts. 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 the results exceed the acceptable level.
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.

Troubleshooting: DO NOT REUSE or RETURN QwikCheck beads to the container after use. Always use a new and separate testing capillary for testing EACH level of beads.

If the test results do not fall into the target range specified by the QwikCheck™ beads the problem could be:

1. Liquid handling problems:
   - QwikCheck-beads have not been thoroughly mixed, or air bubbles are present.
   - The SQA capillary or counting chamber was not prepared properly with the beads or negative control.
   - The QwikCheck-beads have expired, are contaminated, mixed with old/other level of samples or the bottle cap is not closed tightly.
   - The same testing capillary was used for different levels of beads (re-use of testing capillary).
2. Material and subjective factors:
   - The wrong target value has been referenced (in the set-up) for the type of counting chamber/system
   - The counting chamber is inaccurate due to damage or age
   - A calculation or dilution error has been made by the operator (subjective error)

Recommendation: Verify that the QwikCheck-beads have not expired. Run new samples after reviewing the directions. Follow the directions for each type of counting chamber. If the target values are still not met, contact the manufacturer for support.

Limitations: QwikCheck™ beads cannot be used to perform positive quality control for motility or correct for technician errors or faulty equipment.

References: WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th Edition, WHO, 2010.