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QwikCheck™beads

A QUALITY CONTROL MATERIAL FOR AUTOMATED AND MANUAL SPERM COUNTING SYSTEMS

Introduction and Intended Use:

QwikCheck™ beads is an external quality control material for use in automated and manual sperm counting systems. The material is for in-vitro use only and is to be used as a tool to assess the accuracy and precision of the laboratory's sperm counting methods by providing a known target value and +/- range. Although the beads were developed for use on the SQA-V automated and visualization system, they can be used for manual proficiency testing on hemacytometers such as Neubauer counting chambers such as Makler, and conventional fixed coverslips.

QwikCheck™ beads is supplied in a kit containing two vials of known concentrations of 4-micron latex beads suspended in an aqueous solvent and one vial of negative concentration and motility control (diluent). The beads are run in the same manner the laboratory runs sperm counts on the SQA-V, manual counting chambers and the SQA-V visualization system.

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** be run on the SQA-V automated and visualization systems prior to each day of semen analysis testing.

For in-vitro use only:

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 at room temperature (20-25 ºC or 65-77° F). The expiration date assumes that **QwikCheck™ beads** are stored at room temperature in their original containers and tightly capped to prevent evaporation.

Warning:

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

Basic Instructions for using QwikCheck™ beads

1. Gently mix by rotating beads by hand (do not use a vortex) the QwikCheck™ beads prior to use in order to distribute the beads evenly in the suspension. It is imperative that the beads are evenly mixed in order to insure the accuracy of the target value.
2. The negative control material does not require extensive mixing.
3. Immediately withdraw a sample of the control material after opening the container.
4. Immediately and tightly close the container after withdrawing a sample to avoid evaporation or spillage.
5. Follow the same procedure normally used for manual or automated semen analysis (see detailed instructions below).

Target Value and +/- Ranges

Each batch of QwikCheck beads has established Target Values and +/- Ranges unique to the batch. Each box and each control bottle is labeled with these Target Values and Ranges. In addition, the MES website provides the Batch Release Forms by batch # with these values. Access this information as follows: www.mes-ltd.com. Enter the website and click on the SUPPORT tab. Then click on QwikCheck Products > QwikCheck Beads. Find the relevant information by batch number.

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

SQA-V Automated System:

1. Refer to the SQA-V User Guide "CONTROLS" section for an explanation of how to set-up the SQA-V to test automated Level 1/Level 2 QwikCheck™ beads. Follow the instructions and screen prompts in the "Controls" section of the SQA-V User Guide.
2. Follow the basic instructions for mixing and preparing "QwikCheck™ beads" noted previously.
3. Aspirate a sample of the beads or negative control into the SQA-V capillary in the same manner you would fill the capillary for a normal volume specimen. Make sure that the cuvette section of the SQA-V capillary is completely full of liquid and free of bubbles.
4. Following the SQA-V on-screen instructions for "Controls" insert the SQA-V capillary into the SQA-V in the same manner you would test a normal sample of semen.
5. Print and save Control test results.

SQA-V Visualization System using a standard slide:

1. Refer to the SQA-V User Guide "CONTROLS" section for an explanation of how to set-up the SQA-V to test Manual Level 1/Level 2 QwikCheck™beads or negative control. Follow the instructions and screen prompts as outlined in the "Controls" section of the SQA-V User Guide.
2. Follow the basic instructions for mixing and preparing "QwikCheck™ beads" noted previously.
3. Refer to the SQA-V User Guide "Operating the Visualization System" to understand how to use a standard slide in the SQA-V.
4. Pipette 10 uL of QwikCheck™ beads or negative control onto a standard slide, cover with a 22x22 mm coverslip to provide a 20-micron sample depth.
5. If air bubbles or liquid spillage occurs, a new slide must be prepared to ensure an accurate reading.
6. Insert the standard slide into the visualization chamber, press Zoom-Out all the way to set the magnification at x300 and FREEZE the image using V-Sperm software on the computer.
7. Count the beads manually in the same manner you would count a semen sample, following WHO procedure: Duplicate counts of at least 200 beads (turn the slide adaptor knob to bring multiple fields into view).
8. Each bead on the SQA-V screen (V-Sperm III or the SQA-V screen) represents 1 M/ml – multiple screens must be viewed to reach a minimum count of 200 beads so be sure to divide the final count by the number of screens viewed.
9. Refer to table 2.6 of the WHO Manual 4th Edition to determine if the duplicate counts are acceptable.
10. Enter the results in the SQA-V when prompted.

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

Following the manufacturer's instructions for use of the Neubauer hemacytometer and the WHO Manual Guidelines for assessing sperm concentration (WHO Manual, Section 2.5.2, 4th Edition):

1. Follow the basic instructions for mixing and handling QwikCheck™beads or negative control noted previously.
2. Dilute the high-level QwikCheck™beads 4 times and the low-level QwikCheck™beads 2 times using distilled water.
3. Use the negative control material as is.
4. Secure the coverslip on the counting chamber.
5. Transfer approximately 10 µl of the diluted control sample or negative control to each of the counting areas of the hemacytometer.
6. Incubate the hemacytometer for about 5 minutes in a humid chamber (the beads will sediment).
7. Count the beads at a magnification of x200 – x400 in 5 large squares.
8. Run duplicate counts of at least 200 beads and check to see if the results are statistically relevant by calculating the sum and difference of the two counts. Refer to table 2.6 of the WHO Manual, 4th Edition for validation. Re-run new samples if there is a counting error that exceeds the acceptable level.
9. Add the duplicate counts together and divide by 2 to get an average of the two counts. Divide this number by 5 for high-level and by 10 for low-level QwikCheck-beads. **Example: For an average count of 230 on a 1:4 dilution and 5 squares counted per chamber, the conversion factor is 5 and the bead concentration 230 / 5 = 46 X 10^6 / ml.**
10. Compare the results to the QwikCheck™ beads target values.

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. Follow the basic instructions for mixing and handling QwikCheck™ beads noted previously. 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 QwikCheck™ beads or negative control in the center of the lower disc.
4. Place the cover glass on the four tips – this will disperse a uniform 10-micron thick bead sample over the lower disc.
5. Set focus at X200. Locate the grid in the center of the view area.
6. Run duplicate counts of at least 200 beads and check to see if the results are statistically relevant by calculating the sum and difference of the two counts. Refer to table 2.6 of the WHO Manual, 4th Edition for validation. Re-run new samples if there is a counting error that exceeds 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, fixed coverslip, requires no dilution, the field of view of the microscope 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 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. Follow the basic instructions for mixing and handling QwikCheck™ beads or negative control noted previously. Sample dilution is not usually required for fixed coverslip type counting chambers.
3. Load the chamber with 3-5 µl of beads or negative control from the two sides of the fixed coverslip type chamber.
4. Set the focus at 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. Check to see if the results are statistically relevant by calculating the sum and difference of the two counts. Refer to table 2.6 of the WHO Manual, 4th Edition for validation. Re-run new samples if there is a counting error that exceeds 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: QwikCheck beads are not to be re-used or expelled back into 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:
   - The QwikCheck-beads material is not thoroughly mixed or air bubbles are present.
   - The SQA-V capillary or counting chamber was not prepared properly with the beads or negative control.
   - The QwikCheck-beads have expired or the bottle cap is not closed tightly.
   - Use a new and separate testing capillary for testing each control level to prevent mixing/contaminating the material and causing errors.

2. Material and subjective factors:
   - The wrong target value has been referenced 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. Re-run the 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

1. QwikCheck™ beads cannot be used to perform positive quality control for motility.
2. QwikCheck™ beads cannot correct technician errors or errors caused by faulty equipment.

References: WHO Laboratory Manual for the Examination of Human Semen, 4th Edition, Cambridge University Press, 1999, Reprinted 2000.