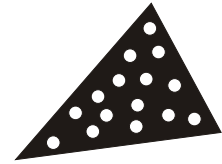


accu-beads®

18 M/ml and 35 M/ml



A Quality Control Check for Automated and Manual Sperm Counting Methods

IMPORTANT NOTE: *The accu-beads bottle caps must be tightly screwed on to prevent evaporation of solution, which will result in increased bead concentrations.*

Summary and Principle

Accu-beads is a quality control product developed to validate the accuracy of automated and manual methods of sperm counting. In accordance with CLIA regulations, **accu-beads** is a two level test. Each kit contains two known concentrations of latex beads (18 M/ml and 35 M/ml) suspended in an aqueous solution. The **accu-beads** solution is used in the same manner normally used within your laboratory for standard sperm counting.

For automated methods (e.g. semen analyzers), **accu-beads** test and evaluate the performance of the optical system and the resulting computer image processing. For manual methods, **accu-beads** test technician techniques and counting practices. Under both circumstances, the precision of the chamber used is also tested.

The **accu-beads** quality control check should be performed each day prior to the evaluation of actual sperm samples. In this way, any discrepancies are identified and corrected beforehand, preventing the loss of the day's testing due to erroneous technical or counting procedures.

Reagents: For in-vitro diagnostic use only.

Each kit contains two bottles of a 5 ml latex bead suspension.

One bottle contains a bead concentration of 18 M/ml.

One bottle contains a bead concentration of 35 M/ml.

The accu-beads kit should be stored at room temperature.

The expiration date listed on the bottles applies to the products in their intact containers when stored as directed.

Warning: Contains 0.1% Sodium Azide. All other ingredients are not harmful in the concentration provided. For more information, refer to HTR Material Safety Data Sheet #093010.

Preparation of Accu-beads

1. Vortex or shake the accu-beads bottles thoroughly to promote even distribution of the beads.
2. The accu-beads should be used in the same manner which you would normally use for standard sperm counts (e.g. volume, dilution, counting method).
3. Immediately open the cap and pipette the required volume (follow the chamber manufacturer's instructions) of bead solution onto the counting chamber. If using a hemacytometer, a 1:20 dilution must be made.
4. Wait at least 20 seconds to allow to settle. Hemacytometers may require longer.
5. Place the counting chamber on the analyzer or microscope stage.
6. Examine the sample for uniform bead distribution.

For HTM-S and HTM-2000 Counting Procedures, see back of page.

Manual Counting Procedures

1. Count the beads according to your standard counting procedure.
2. When using an eyepiece reticle for counting, at least 10 squares in 5 different fields should be counted. The minimum total number of beads counted should be 200. For a higher degree of accuracy, count more fields.
3. When using a Makler chamber with a gridded coverslip, a hemacytometer, or a gridded Cell-Vu, follow the chamber manufacturer's counting instructions.
4. Calculate the bead concentration according the chamber manufacturer's instructions.
5. Count another aliquot of the same sample. The results should be within 10% of each other to be considered valid.
6. If the results are valid, average the two counts and compare to the accu-beads acceptable ranges listed below.
7. The counting procedure above should be performed with both accu-beads concentrations.
8. Record all results along with pertinent information such as the chamber used and the name of the person performing the QC procedure.

Acceptable Accu-beads Ranges

For 18 M/ml Concentration: between 15.5 and 20.5 M/ml

For 35 M/ml Concentration: between 30 and 40 M/ml

Manual Troubleshooting

1. Concentrations too high or too low.
 - a. Mixing error: Mix accu-beads again for even distribution.
 - b. Pipetting error: Reload the chamber being careful to avoid overloading or underloading the chamber.
 - c. Observer error: Have a different technician confirm counting accuracy.
 - d. Chamber error: Repeat the QC procedure using another chamber.

Limitation of the Method

1. Accu-beads cannot be used to perform quality control on determining motility accuracy.
2. Accu-beads cannot correct inaccurate chamber depths. Adjustments will have to be made within your laboratory by evaluating the reproducibility of your results.
3. Validation of bead concentrations should only be performed using a hemacytometer.
4. Accu-beads cannot correct technician error.

Using Accu-beads for QC of HTM Analyzers

Note: Do not dilute the accu-beads. The accu-beads solutions are designed to be used directly from the bottle for analysis with the HTM analyzers. Diluting the solutions may introduce sampling errors.

Preparation of HTM-S and HTM-2000

1. From the MAIN MENU select MAIN GATES.
2. Choose a parameter set that is not in use by your laboratory for other studies.
3. Since the accu-beads will be used on a regular basis, it is best to designate one parameter set for the QC process.
4. Change the parameter settings to match the guidelines in the chart.
5. Enter the NOTEPAD.
6. Check that there is no DILUTION entered. You may also wish to enter the name of the person performing the analysis in the NOTEPAD.
7. From the MAIN MENU, select ANALYZER SETUP.
8. Change the image type from PHASE CONTRAST to INFRARED.
9. Open the side panel of the analyzer by turning the four screws counterclockwise.
10. Locate the phase plate (refer to manual).
11. Gently rotate this plate counterclockwise towards the ceiling so it is out of the path of the beam. It should snap into place.
12. Replace the side panel loosely.
13. Note: After the QC Check has been performed, return the image type to PHASE CONTRAST and return the phase plate to its original position.

GUIDELINES FOR PARAMETER SETTINGS

	HTM-S HTM-2000
FRAMES	5
FRAMES RATE	20
MINIMUM CONTRAST	7
MINIMUM SIZE	5
NON-MOTILE HEAD SIZE	12
NON-MOTILE INTENSITY	20
SIZE LIMITS OR GATES	0.5 to 2.2
INTENSITY LIMITS OR GATES	0.5 to 1.6

NOTE: These are guidelines only. Adjustments may be required.

Performing the Accu-bead Analysis (all HTR Analyzers)

1. Focus the image clearly.
2. Select 1 field for analysis and press START SCAN.
3. When the analysis is complete, view the PLAYBACK screen.
4. Check that nearly all beads are labeled properly.
5. If optimum labeling is not achieved, refer to the analyzer manual for QC procedures on the identification of Non-Motile cells.
6. When the labeling is satisfactory, select at least 5 fields (200 cells) for analysis. Record, print or store the concentration results.
7. Perform a second analysis from an aliquot of the same sample. The results should be within 10% of each other to be considered valid.
8. If the results are valid, average the two counts and compare to the accu-beads acceptable ranges on the reverse side.
9. The QC procedure above should be performed with both concentrations of beads.

Troubleshooting

1. Concentration too high or too low:
 - a. Check the dilution factor on the INFO screen.
 - b. Check the Magnification setting.
 - c. Review PLAYBACK to check proper labeling.
 - d. Check/adjust Cell Size and Intensity (Non-Motile Head Size and Intensity) as described in the manual.
 - e. Repeat analysis with new chamber.
 - f. *See also: Troubleshooting for Manual Counting Procedures.*
2. Motility is detected:
 - a. Wipe excess fluid off chamber, wait 1 minute and reanalyze.
 - b. Stage movement may cause slight movement of beads. To prevent this, use the Add Scan feature.
 - c. Set Slow Cells to Static or Non-motile.
3. Image is poor:
 - a. Adjust focus.
 - b. IVOS, CEROS, MASTER C, HTM-C
 - 1) Check ILLUMINATION.
 - 2) Ensure that the 10 NH phase contrast objective is in place.
 - c. HTM-S, HTM-2000
 - 1) Check that IMAGE TYPE is set to INFRARED.
 - 2) Check that the PHASE PLATE is positioned properly.

For More Information: Please visit www.hamiltonthorne.com/documentation/ or refer to the chamber manufacturer's instructions.

For Customer Service: E-mail: sales@hamiltonthorne.com; call: (978) 921-2050; or fax: (978) 921-0250.



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