



## 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 sperm counting methods. In accordance with CLIA regulations, **accu-beads** is a two level test. Each kit contains two known calibrations of accu-beads solution. The **accu-beads** solution is used in the same manner normally used within your laboratory for standard sperm counting.

The **accu-beads** solution consists of small latex spheres (4  $\mu\text{m}$  in diameter) suspended in an isopycnic medium. The physical properties of this medium allow the beads to float and promote even distribution of the beads throughout the solution.

**Accu-beads** were designed primarily for use with capillary-loading chambers (e.g. Leja chambers) for validation of automated sperm counting methods (CASA). However, they may also be used for validation manual sperm counting methods using the hemacytometer.

For CASA counting methods, **accu-beads** test and evaluate the performance of the optical system and the resulting computer image processing. For manual counting 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 5 ml latex bead suspension.

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 0.07% Sodium Azide. All other ingredients are not harmful in the concentration provided. For more information, refer to the accu-beads Material Safety Data Sheet (available on our web site).

### Preparation of Accu-beads

1. Vortex or shake the accu-beads bottles thoroughly (30-45 seconds) 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. Open the cap and immediately pipette the required volume (follow the chamber manufacturer's instructions) of bead solution into the counting chamber. If using a hemacytometer, a 1:19 (bead to  $\text{H}_2\text{O}$ ) mixture must be made. This gives a 1 in 20 dilution.
4. Close the bottle.
5. Wait at least 1 minute to allow the accu-beads to settle. Hemacytometers require 5 minutes.
6. Place the counting chamber on the analyzer or microscope stage.

### Acceptable Accu-beads Ranges

#### Fixed Coverslip Chambers

- Vial #1: 35 M/ml calibration: between 30 and 40 M/ml
- Vial #2: 18 M/ml calibration: between 15.5 and 20.5 M/ml

#### Hemacytometer

- Vial #1: 46 M/ml calibration: between 39 and 53 M/ml
- Vial #2: 23 M/ml calibration: between 19 and 27 M/ml

### Instructions for Automated Counting Procedures: see back of page.

### Instructions for 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 above.
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.

### 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 of known depth.

### 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. Diluting the solutions may introduce sampling errors.*

### Preparation of CASA II Software

1. From the MAIN screen, select SETUPS.
2. Create or select the accu-beads setup.
3. Set the parameter settings to the guidelines in the below chart.
4. Ensure that the appropriate chamber and depth have been selected.
5. Make sure that the 10x NH phase contrast objective is in place.
6. Verify the proper magnification.
6. Perform a Live Calibration and set the illumination. The accu-beads should be illuminated blue.

CASA II SYSTEMS	Version 1
Frame Count	5
Frame Capture Speed (Hz)	60
Elongation Max	100
Elongation Min	1
Head Brightness Max	150
Head Size Max	100
Head Size Min	8
Static Require Tails	No
Capillary Correction	1

### Preparation of Version 12 & 14 IVOS and CEROS

1. From the MAIN MENU, select SETUP.
2. Choose an ANALYSIS SETUP that is not in use by your laboratory for other studies and name it **accu-beads** (or something similar). Since the accu-beads will be used on a regular basis, it is best to designate one ANALYSIS SETUP solely for the QC process.
3. Change the parameter settings to the guidelines in the chart.
4. Press STAGE SETUP. Select the appropriate chamber and depth.
5. Make sure that the 10x NH phase contrast objective is in place.
6. Press OPTICS SETUP. Verify the proper MAGNIFICATION. Set ILLUMINATION for a PHOTOMETER reading of 50 - 55.

LEGACY CASA SYSTEMS	Versions 10-14
Frames / No. of Frames	5
Frame Rate / Frames/sec	60
Minimum Contrast	50
Minimum Size	3
Non-motile Head Size / Cell Size	5
Non-motile Intensity / Cell Intensity	140
Size Limits or Gates	0.5 to 8.0
Intensity Limits or Gates	0.5 to 2.0
Elongation Limits	20 to 100

**NOTE: Provided settings are guidelines only. Adjustments may be required.**

### Preparation of Version 10 IVOS, CEROS and MASTER-C

1. From the MAIN MENU, select SETUP.
2. Choose an ANALYSIS SETUP that is not in use by your laboratory for other studies and name it **accu-beads** (or something similar). Since the accu-beads will be used on a regular basis, it is best to designate one ANALYSIS SETUP solely for the QC process.
3. Change the parameter settings to the guidelines listed in the chart.
4. Press CONFIGURE STAGE. Select the appropriate chamber and depth.
5. Make sure that the 10x NH phase contrast objective is in place.
6. Press CALIBRATE OPTICS. Verify the proper MAGNIFICATION. Set ILLUMINATION for a LOW PHOTOMETER reading of 50 - 55.

### Performing the Accu-bead Analysis (all HTR Analyzers)

1. Focus the image clearly.
2. Select 1 field for analysis and analyze one field.
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 analysis parameters 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. CASA II: Adjust Slow and Static VAP/VSL.  
Legacy Systems: Set Slow Cells to Static or Non-motile.
3. Image is poor:
  - a. Adjust focus.
  - b. Check ILLUMINATION.
  - c. Ensure that the 10 NH phase contrast objective is in place.

**For copies of Certificates of Analysis and additional information about accu-beads, please visit: [www.hamiltonthorne.com](http://www.hamiltonthorne.com)**

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



**HAMILTON THORNE INC.**

100 Cummings Center, Suite 465E, Beverly, MA 01915  
[info@hamiltonthorne.com](mailto:info@hamiltonthorne.com), [www.hamiltonthorne.com](http://www.hamiltonthorne.com)