BECKMAN COULTER
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# The new Avanti J-15 centrifuge improves sample protection and maximizes sample recovery

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## Abstract

Sample protection is the ultimate goal of any workflow that aim to provide quality specimen for downstream processes. Often, centrifugation is the first step in processing biological and chemical materials. Purity and adequate amounts of sample influence the scientific outcome by improving the quality of the data and minimizing the need for replications, which reduces down time. The new Avanti J-15 series centrifuges by Beckman Coulter were designed with this customers need in mind. The implementation of Ultra Harmonic Technology into the Avanti J-15 series renders our centrifuge ideal for the processing of samples that require special deceleration profiles during pelleting or separation. By focusing on sample protection, the Avanti J-15 series has reduced sample disturbance during acceleration and deceleration, increasing sample yield, decreasing contamination and increasing centrifugation efficiency.

## Introduction

Centrifugation is an essential and primary step in many biological and chemical workflows. It is used in a wide array of procedures including tissue culture, nucleic acid isolation, blood processing, protein purification, virus isolation, transfection and subcellular fractionation. For 70 years, Beckman Coulter has produced high quality centrifuges that have aided the progress of countless scientific discoveries. As traditional tabletop centrifugation primarily considers time, temperature and speed as pillars of centrifuge design, Beckman Coulter is revolutionizing the market with the newly designed Avanti J-15 series centrifuges that maximize the efficiency and yield of sample separation and pelleting with the implementation of Ultra Harmonic Technology.

During deceleration multiple forces act on a sample, which can result in sample disturbance. The Ultra Harmonic Technology optimizes sample separation by reducing the net changes of forces to the sample, maximizing the amount of specimen presence in the pellet and reducing the presence of contaminants or unpelleted materials in the supernatant. The efficiency of separation is significantly important for samples that form soft pellets, less adherent and less compact, and require modified protocols to coast (no break profile) during deceleration. The experiments were conducted to examine the relative pelleting efficiencies of the Avanti J-15 series versus current table top instruments in the market, during the preparation of electrocompetent *E. Coli* DH5-alpha bacteria cells.

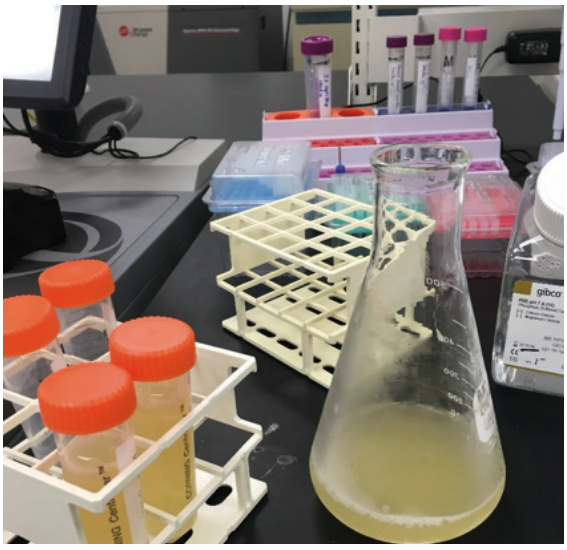

**Figure 1.** Experimental set up and aliquots of bacteria sample.

## Materials

*E. Coli* DH5-alpha competent cells (cat. C2989K) was purchased from New England BioLabs. Glycerol solution (cat. 536407) was purchased from Sigma-Aldrich.

Avanti J-15R (P/N B99517, B99516, B99515, B99514) equipped with JS-4.750 rotor (P/N B77580), and Allegra 14R[§] (P/N A99465) equipped with SX4750 rotor (P/N 392806) were from Beckman Coulter.

## Methods

*E. Coli* DH5-Alpha was grown overnight at 37° C while shaking at 170 RPM. Following overnight growth, the cells were allowed to chill in a 4° C water bath. A sample of the culture was collected for optical density measurement ($OD_{600}$) using a Beckman-Coulter DU730 UV/Vis Spectophotometer with a 10mm pathlength. The sample was diluted at a 1:3 ratio

The 40 mL of cells were aliquot in 50 mL conical tubes and one sample was assigned to each instrument, Avanti J-15R, Allegra X-14R and a competitor unit (Fig. 1).

Each instrument was configured to spin at 3,600 x *g* for 20 minutes with maximum acceleration and deceleration profiles for initial cell pelleting. Each pellet was washed in 20 mL of 10% glycerol and spun at 3,600 x *g* for 20 minutes using profile 4 during acceleration and deceleration to prevent sample loss. After centrifugation, the supernatant was carefully removed and glycerol wash step was repeated twice. Following the last centrifugation step, the cells were resuspended in 20 mL of 1x PBS. An aliquot was diluted at 1:3 with 1X PBS and the $OD_{600}$ was measured using Beckman-Coulter DU730 UV/Vis Spectophotometer and recorded.

## Results and Discussion

To explore advances of the Ultra Harmonic Technology over the current models, identical initial amount of samples were prepared and processed under identical settings in all three units. Table 1 summarize our findings. While the OD was measured at 1:3 dilution, the cell number reported is normalized for dilution. Following cell growth, the initial optical density of the cells was measured and the cell density was calculated to be $1.35 \times 10^9$ cells/mL. The density calculation (genomics.agilent.com) uses the extinction coefficient for *E. Coli* from the $OD_{600}$ reading taken with a spectophometer.

The cell recovery at the end of three glycerol washed was empirically calculated and compared across all three units. The result on Table1 and it clearly demonstrate the Ultra Harmonic Technology aids in sample protection and maximum recovery.

The new Avanti J-15 instrument performed with superior results that demonstrate to improve sample separation and pelleting efficiencies compared to current models. After multiple glycerol washes, only less than 2% of the sample was loss. This result is not only mathematically determined, but also visually observed. The sample retrieved from the Avanti J-15 instrument after the pelleting step has a clear supernatant, in contrast with the Allegra X-14R (Figure 2).

The use of a competitor instrument resulted in almost 50% sample loss, which can significantly impact downstream workflows. By achieving less than ideal sample recovery, the amount of starting materials is depleted leading to laborious steps of repeating an experiment. Inadvertently, the downfall of repetition leads to increase cost and divergence of resources and laboratory personnel.

Interestingly, the Beckman Coulter Inc. Allegra X-14R performed at a threefold efficiency compared to competitor instruments with significant sample protection.

| Instrument | OD Measurement (1:3 dilution) | Cell Recovery (cells/mL) (normalized for dilution) | Sample Loss |
|---|---|---|---|
| Initial Cell Culture | 0.56 | $1.35 \times 10^9$ | N/A |
| Avanti J-15R | 0.55 | $1.33 \times 10^9$ | 1.6% |
| Allegra X-14R | 0.48 | $1.15 \times 10^9$ | 14% |
| Competitor | 0.30 | $0.73 \times 10^9$ | 46% |

**Table 1**

Not all samples pellet the same and with the same efficiency. These samples are considered soft pellet material because they do not compact well. Soft pellet samples are prone to sample loss and are usually spun with longer acceleration and deceleration profile to prevent sample disturbance and maximize yield. Soft pellets are present in nature (i.e. algae) or they can be created. The process of production of electrocompetent cells involves a number of cell washes with glycerol. The glycerol is cryopreserving and known to maintain osmolarity and increase electro transformation efficiency. Curiously, during the glycerol washing process, bacteria cells also lose adherence and they are centrifuged in modified protocols that require acceleration and deceleration profiles at 4, which decrease pellet disturbance.

## Conclusion

Sample protection and maximum yield is of crucial importance to research. With sample loss, an experiment may need to be repeated multiple times. The inadequate separation of samples from contaminants may lead to inaccurate results. The Ultra Harmonic Technology refines acceleration and deceleration and improves the quality of sample separation. Therefore, the new Avanti J-15 series is poised to circumvent sample loss problems and maximize sample yield.

The Avanti J-15 series instruments were designed with the Ultra Harmonic Technology. It incorporates important features of acceleration and deceleration, present in refined ultracentrifugation, into tabletop centrifugation, therefore reducing sample disturbance during these critical times. This technology propels routine tabletop centrifugation, beyond time, temperature and speed. The Ultra Harmonic Technology maximizes sample recovery. Under the same instrument settings, the competitor technology was unable to obtain the sample protection observed in our models. The Avanti J-15 series showed an improved acceleration and deceleration profile that increases sample integrity and protection.

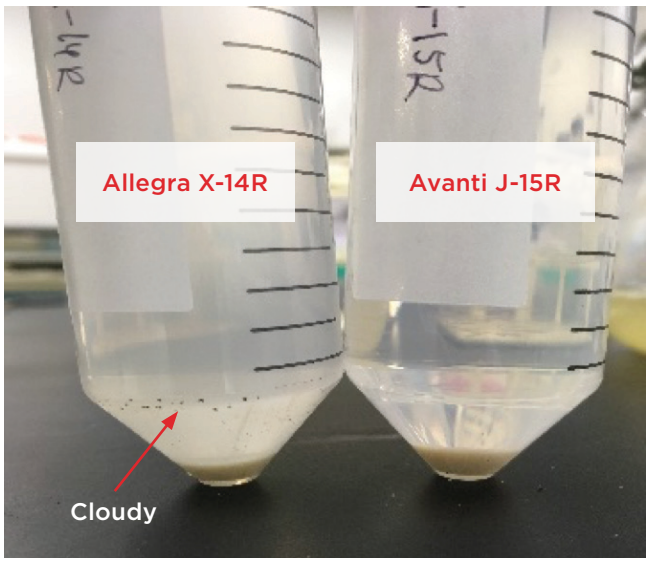


**Figure 2:** Visual comparison between Allegra X-14R and Avanti J-15R sample.