

# Comparison of Mechanical Tissue Grinders and the Mini Beadbeater 96 for Homogenization of Murine Lung and Thigh Tissue for Subsequent Quantitation of Bacterial Density

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## Abstract

### Background

Quantitation of bacterial density in animal tissues is commonplace in pre-clinical antibacterial drug discovery and development research. Routinely used tissue homogenization methods are labor intensive, time consuming processes that can lead to repetitive motion related injuries to laboratory personnel.

### Findings

In this study, we compared our standard method of tissue homogenization, mechanical tissue grinders, to the Mini Beadbeater 96 for the homogenization of murine lung and thigh tissue from standard neutropenic infection models. The Mini Beadbeater 96 proved to be an effective method for homogenizing murine lung and thigh tissues for subsequent bacterial quantitation, with results comparable to those obtained with mechanical tissue grinders.

### Conclusion

This method proved to be a practical, high throughput, time efficient, cost effective, ergonomically friendly method for tissue homogenization.

**Keywords:** Beadbeater; Tissue homogenization; Tissue grinders

**Abbreviations:** Beadbeater : Mini-Beadbeater 96; BAP: Trypticase soy agar with 5% sheep blood; MSDs: musculoskeletal disorders; STA56, ATCC29213: *Staphylococcus aureus*; KP462: *Klebsiella pneumoniae*; PSA1401, PSAJJ-1-29: *Pseudomonas aeruginosa*

## Introduction

Animal infection models are routinely used to evaluate the efficacy of antimicrobial agents. Since these models are considered predictive of clinical success, data generated from these models are used by regulatory agencies in approval processes and setting susceptibility breakpoints [1,2]. Given the general utility of these models to evaluate anti-infective therapies, their use is commonplace in antibacterial drug discovery and development research. Specific models of interest for understanding the pharmacokinetics and pharmacodynamics of antibacterials include murine lung and thigh infection models. To extract bacteria from these infected animal tissues, homogenization is required prior to quantitation of bacterial density. Although the method for tissue homogenization is not always specified in the literature, commonly

used methods for a variety of endpoints include mechanical tissue grinders (generators; our standard method) [3,4], hand held Dounce homogenizers, reinforced polyethylene bags [5,6] and a Stomacher® [7,8]. Although each of these methods can effectively homogenize rodent tissues, they are labor-intensive, time consuming processes often requiring cleaning and sterilization steps between samples. Moreover, many laboratory methods are repetitive in nature and thus have the potential to cause injury to personnel over time [9,10]. Thus, any methods that Beadbeater techniques have been used to disrupt plant tissue prior to polymerase chain reaction detection of plant pathogens [11] and homogenize mouse tissue samples prior to LC-MS/MS detection of drug concentrations in these tissues

[12]. Samples are efficiently homogenized by violent shaking (beating) for a specified period of time using the Beadbeater technology. Prior experience (LML) and preliminary experiments within the Center for Anti-Infective Research and Development (data not shown), determined that the Mini-Beadbeater 96 (Beadbeater) could effectively homogenize murine lung and thigh tissue required for subsequent quantitation of bacterial density. In this study we evaluated the efficiency and effectiveness of the Mini Beadbeater 96 as compared to our standard method of tissue homogenization of murine lung and thigh samples.

## Materials and Methods

### Bacterial strains

Two *Staphylococcus aureus* strains (STA56, ATCC29213), one *Klebsiella pneumoniae* strain (KP462) and two *Pseudomonas aeruginosa* strains (PSA1401, PSAJJ-1-29) were used in these studies. All strains were maintained at -80°C in skim milk and subcultured onto Trypticase soy agar with 5% sheep blood (BAP) prior to use.

### Animals

Specific-pathogen free female ICR (CD-1) mice (25-30g) or Balb/c mice (17-20g) were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). All mice were provided food and water *ad libitum*. This study was reviewed and approved by the Hartford Hospital Institutional Animal Care and Use Committee.

### Murine Thigh Infection Model

Mice were rendered transiently neutropenic by intraperitoneal injections of cyclophosphamide (Baxter, Deerfield, IL or Sigma-Aldrich, St. Louis, MO) at 150 mg/kg and 100 mg/kg of body weight given 4 days and 1 day, respectively, prior to inoculation. Bacterial suspensions of *S. aureus*, *K. pneumoniae* and *P. aeruginosa* were prepared in sterile normal saline. For each bacterial isolate tested, 2 groups of mice (n=3) were inoculated intramuscularly in each thigh with 0.1 ml containing approximately 10<sup>7</sup> CFU/ml in sterile normal saline. At 2h post-infection, corresponding to our in-house procedure for the onset of therapy, 2 groups of mice (n=3) from each isolate group were euthanized by CO<sub>2</sub> asphyxiation and cervical dislocation. Both thighs from each animal were harvested aseptically and individually homogenized by one of two methods: (1) Generator (10 x 115 mm, Pro Scientific Inc. Oxford, CT) in 14 ml polypropylene tubes (BD Falcon, Pittston, PA) with 5ml sterile normal saline to be consistent with historical homogenization volumes, or (2) Beaten in the Beadbeater (purchased from BioSpec Products, Bartlesville, OK) for 2 minutes in 5 ml polypropylene tubes (Denville Scientific, South Plainfield, NJ) with 5 ml sterile normal saline and 5 stainless steel beads (3.2 mm; BioSpec Products). Each sample was then passed through a large pore filter (280µ) to remove fibrous tissue (Interscience Bagpage+, Cole-Parmer). Serial dilutions of filtered thigh homogenates were cultured onto BAP for quantitation of bacterial density. Data outliers were identified using an interquartile range method and removed from the group mean.

### Murine Lung Infection Model

Mice were rendered transiently neutropenic by intraperitoneal injections of cyclophosphamide (Baxter, Deerfield, IL or Sigma-Aldrich, St. Louis, MO) at 250 mg/kg and 100 mg/kg of body weight given 4 days and 1 day, respectively, prior to inoculation. Bacterial suspensions were prepared in sterile normal saline for *K. pneumoniae* and *P. aeruginosa* isolates or in 3% hog gastric mucin for *S. aureus* isolates. For each bacterial isolate tested, 4 groups of mice (n=6) were inoculated. Under 2% isofluorane anesthesia, 0.05ml of the bacterial suspension containing approximately 10<sup>7</sup> CFU/ml was instilled orally and nares were blocked. The mice aspirated the suspension into the lungs while being held vertically for approximately 60 seconds. At 2h (KP, PSA) or 3h (STA, ATCC) post-infection, 2 groups of mice (n=6) from each isolate were euthanized by CO<sub>2</sub> asphyxiation and cervical dislocation. During studies evaluating a bead-free matrix (section 2.4.4 below), an additional two groups of animals were sacrificed 24 hours later. After euthanization, whole lung tissues from all animals were harvested aseptically and individually homogenized as described below in Sections 2.4.1 and 2.4.2. To be consistent with historical homogenization volumes, lung tissues were homogenized in 1ml of sterile normal saline. Tissue harvest time points of 2h or 3h (depending on organism) and 24h post-infection were selected to be consistent with our in-house procedures for onset of therapy and end of study, respectively.

### Homogenization of Murine Lung Tissue with Generators or Beadbeater

Whole lung tissues from each animal were homogenized by one of two methods: (1) generator (7 x 120 mm, ProMulti Gen 7) in polystyrene tubes (12 x 75 mm BD Falcon, Pittston, PA) with 1ml sterile normal saline or (2) beaten in the Beadbeater for 2 minutes in 5 ml polypropylene tubes (Denville Scientific, South Plainfield, NJ) with 1 ml sterile normal saline and approximately 1 ml of zirconia/silica beads (1.0 mm; BioSpec Products). Each sample was then passed through a large pore filter (280µ) (Interscience Bagpage+, Cole-Parmer) and serial dilutions of lung homogenates were cultured onto BAP for quantitation of bacterial density. Bacterial densities outside one standard deviation were excluded from the group mean.

### Homogenization of Murine Lung Tissue in the Beadbeater with and without Zirconia/Silica Beads

We wanted to streamline our tissue processing methodology even further and select one bead type for both lung and thigh tissue homogenization. While standardized use of zirconia/silica beads across models was considered, initial experiments showed that these beads were less effective in homogenizing thigh tissue and thus complete bacterial densities were not recoverable (data not shown). Thus, 3.2mm stainless steel beads currently utilized for thigh homogenization were evaluated for lung tissue homogenization. However, using the stainless steel beads to homogenize lung tissue resulted in cracked tubes and loss of sample

despite modifications to the number of beads per tube, the homogenization times and the saline volume. As such, these studies evaluated the utility of murine lung tissue homogenization in the Beadbeater without using any beads. For these experiments, whole lung tissues from each animal were beaten in the Beadbeater for 2 minutes by one of two methods: (1) with or (2) without zirconia/silica beads in 5 ml polypropylene tubes (Denville Scientific, South Plainfield, NJ) with 1ml of sterile normal saline. Each sample was filtered and cultured as described above. Bacterial densities outside one standard deviation were excluded from the group mean.

## Results

### Murine Thigh Infection Model-Generators vs. Beadbeater

The bacterial density in murine thigh tissue harvested 2 hours post-infection from mice infected with STA56, ATCC29213, KP462 or PSAJJ1-29 and homogenized by generators was  $5.68 \pm 0.11$ ,  $5.72 \pm 0.12$ ,  $6.21 \pm 0.02$  and  $4.81 \pm 0.15 \log_{10}$  CFU, respectively and from thigh tissue homogenized by the Beadbeater was  $5.75 \pm 0.14$ ,  $5.86 \pm 0.07$ ,  $6.40 \pm 0.09$  and  $5.05 \pm 0.17 \log_{10}$  CFU, respectively (Figure 1). Upon visual inspection, the generators homogenized the thigh muscle and bone into a uniform suspension, while the Beadbeater left the bone and some muscle tissue intact. However, the resultant bacterial densities were comparable.

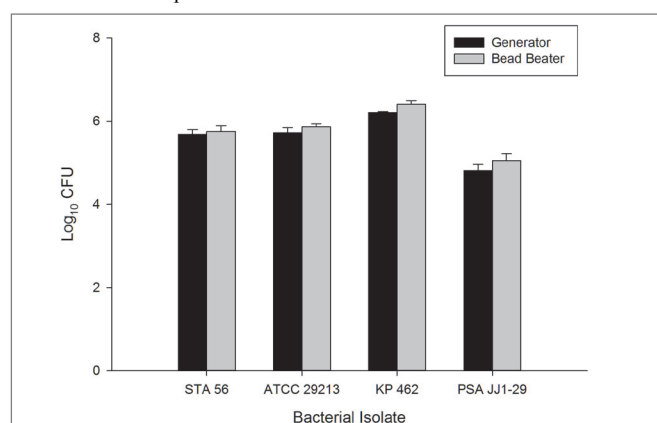


Figure 1 Comparison of bacterial burden in murine thigh tissue 2h post-inoculation homogenized by mechanical tissue generators (black bar) or Mini Beadbeater 96 (gray bar). Bars represent mean ±SD.

### Murine Lung Infection Model-Generators vs. Bead beater

The bacterial density in murine lung tissue harvested 2 hours (KP, PSA) or 3h (STA, ATCC) post-infection from mice infected with STA56, ATCC29213, KP462 or PSA1401 and homogenized by generators was  $5.97 \pm 0.11$ ,  $5.73 \pm 0.15$ ,  $5.83 \pm 0.72$  and  $6.25 \pm 0.39 \log_{10}$  CFU, respectively and from lungs homogenized in the Beadbeater was  $6.19 \pm 0.24$ ,  $6.28 \pm 0.17$ ,  $6.43 \pm 0.30$  and  $6.31 \pm 0.09 \log_{10}$  CFU, respectively (Figure 2). Murine lung tissue processed by both generators and the Beadbeater resulted in completely homogenized, uniform suspensions with comparable bacterial densities.

### Murine Lung Infection Model- Zirconia/Silica Beads vs. no Beads

In two independent experiments, the bacterial density in murine lung tissue harvested 2 hours post-infection from mice infected with PSA1401 and

homogenized with beads was  $5.88 \pm 0.46$  and  $5.71 \pm 0.15 \log_{10}$  CFU in Trial 1 and 2, respectively. The bacterial density in lung samples homogenized without beads was  $6.11 \pm 0.26$  and  $6.13 \pm 0.52 \log_{10}$  CFU in Trial 1 and 2, respectively. The bacterial density in murine lung tissue harvested at 24 hours post-infection and homogenized with beads was  $9.36 \pm 0.11$  and  $9.91 \pm 0.33 \log_{10}$  CFU in Trial 1 and 2, respectively. The bacterial density in lung samples homogenized without beads was  $9.43 \pm 0.11$  and  $9.73 \pm 0.11 \log_{10}$  CFU in Trial 1 and 2, respectively (Figure 3). Lung tissue homogenized in the Beadbeater with and without zirconia/silica beads resulted in completely homogenized, uniform suspensions with comparable bacterial densities.

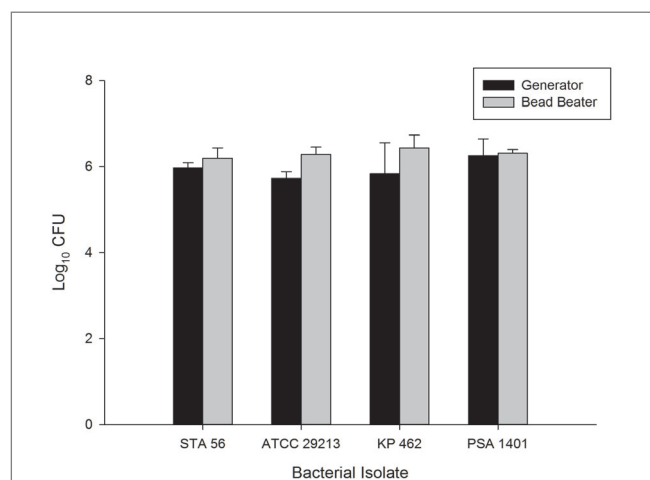


Figure 2 Comparison of bacterial burden in murine lung tissue 2h (KP, PSA) or 3h (STA, ATCC) post-inoculation homogenized by mechanical tissue generators (black bar) or zirconia/silica beads in the Mini Beadbeater 96 (gray bar). Bars represent mean ±SD.

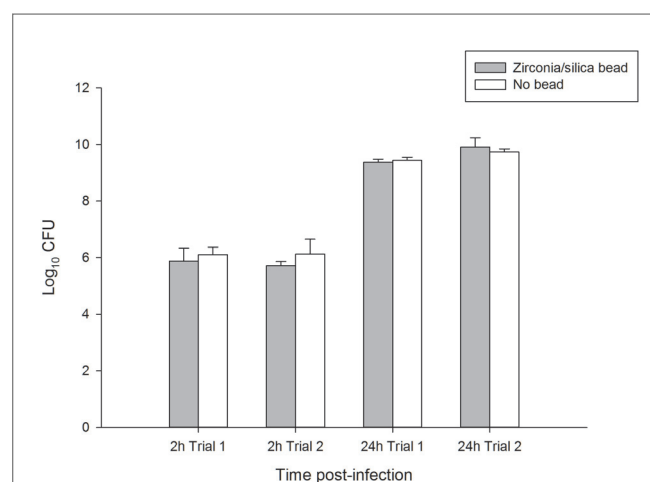


Figure 3 Comparison of bacterial burden of PSA1401 in murine lung tissue 2h and 24h post-inoculation homogenized with zirconia/silica beads (gray bar) or no beads (white bar) in the Mini Beadbeater 96. Bars represent mean ±SD.

## Discussion

Animal infection models are the cornerstone of antibacterial drug discovery and development. Homogenization of tissues from these models is

necessary prior to quantitation of bacterial density. Although a variety of homogenization methods are routinely used, most are labor intensive, time consuming processes with the propensity towards repetitive motions.

We compared generators and the Beadbeater for homogenizing murine lung and thigh tissue by quantitating bacterial density in these tissues. Bacterial densities in infected murine thigh tissue homogenized by generators and the Beadbeater using stainless steel beads were comparable even though the generators produced uniform suspensions and the Beadbeater method left the bone and some muscle intact. Bacterial densities in infected murine lung tissue homogenized by generators and the Beadbeater using zirconia/silica beads were comparable with both methods producing completely homogenized, uniform suspensions. Bacterial densities in murine lung tissue homogenized with and without beads using the Beadbeater were comparable with both methods producing completely homogenized, uniform suspensions. Since tissue homogenization of infected murine lung and thigh tissue by both the generators and the Beadbeater resulted in similar bacterial densities, we concluded the Beadbeater method did not compromise the viability of the organisms tested. Bacterial densities in tissues from murine lung and thigh infection models can vary greatly over a multi-log range depending on the inoculum, time of sampling post-infection and efficacy of the therapeutic agents tested. If the homogenization methods were different we would expect to see greater differences in CFU at 2 or 3 hours post-infection where bacterial burdens are lower. However, bacterial densities were comparable at 2 or 3 hours post-infection as well as at 24h where bacterial densities were higher, suggesting these homogenization methods are comparable.

We noted several advantages to using the Beadbeater rather than generators to homogenize murine tissues. Previously we homogenized tissue samples individually using generators that required cleaning (water and ethanol rinses) between samples. Since we typically homogenize 150-200 tissue samples per experiment, the ability to homogenize multiple samples in a short time frame without additional cleaning steps or preparation of water and ethanol rinse tubes resulted in a considerable reduction in overall preparation and tissue processing time. We now routinely homogenize 12 tissue samples in 2 minutes with the Beadbeater. While use of the Beadbeater, even with differing beads for lung and thigh infection models was advantageous, further refinement of the tissue homogenization process by eliminating beads entirely for lung tissue homogenization resulted in reduced cost of materials and labor.

Many laboratory procedures are repetitive in nature such as capping and uncapping tubes, holding tubes, pipetting and vortexing samples. These repetitive procedures have the potential to cause musculoskeletal disorders (MSDs) and have been identified by OSHA as ergonomic problems [9,10]. By using the Beadbeater, we can eliminate the preparation and use of the tubes that are needed to clean and rinse the generators, eliminate capping and recapping the tubes associated with the cleaning and homogenization process, and eliminate the exposure to vibration associated with the use of generators. These translated into savings in terms of homogenization time, cost of supplies and associated labor and reduced the potential exposure of laboratory personnel to MSDs.

The studies described herein found Beadbeater technology to be an effective method for homogenizing murine lung and thigh tissue for subsequent bacterial quantitation, with results comparable to our historical laboratory standard, mechanical generators. Based on these findings, our lab has adopted this as a practical, cost effective, high throughput, labor and time efficient, ergonomically friendly method for homogenizing murine lung and thigh tissues.

## Competing Interests

The authors declare that they have no competing interests.

## Authors' Contributions

LML, JT-R, JLC and DPN contributed to the conception and design of this study, acquisition and analysis of the data, drafting of the manuscript and critical revision. All authors have read and approved the final manuscript.

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