

ImQuest BioSciences is a preclinical contract research and development company that evaluates the potential of new and novel pharmaceutical products. We specialize in the development of drugs, vaccines and biologic products for the treatment and prevention of infectious disease, cancer and inflammatory disease.

Robert W. Buckheit, Jr., Ph.D.  
Chief Scientific Officer  
rbuckheit@imquestbio.com

## In Vivo Mouse Models of Bacterial Infection Peritonitis-Sepsis Model of MRSA Infection

### Introduction

ImQuest BioSciences has optimized the peritonitis-sepsis mouse model to screen for new antibacterial agents against methicillin-resistant *Staphylococcus aureus* (MRSA). The model's popularity derives from its ease of use with small and inexpensive animals, short-duration experiments with reproducible infections, and simple end-points.

The mouse peritonitis-sepsis model, the first animal model for antibiotic research, was used to demonstrate the efficacy of sulphonamides against *Streptococcus pyogenes* in 1935. Subsequently, the use of the model to study infection and screen antibiotics for activity has been reported in numerous publications.

Two models are commonly used as *in vivo* screens for new antibacterial agents: the peritonitis-sepsis model and the neutropenic thigh model (also optimized by ImQuest).

### Sample Protocol and Results

Methicillin-resistant *S. aureus* strain NRS71 (Sangar 252) was obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA).

Stock inocula were prepared from a logarithmic phase culture as follows.

Two aliquots of the culture were harvested by centrifugation and the bacterial pellets suspended in sterile 0.9% saline containing 4% hog gastric mucin type III (vehicle) (Sigma Aldrich) to yield final concentrations of  $1 \times 10^{10}$  colony forming units(CFU)/mL or  $5 \times 10^8$  CFU/mL.

Additional inocula were prepared by making serial two-fold or five-fold dilutions in 0.9% saline containing 4% hog gastric mucin type III.

The concentration (CFU/mL) of the bacterial inocula was verified to be within 15% of the estimated concentration by plating onto tryptic soy blood agar plates.

Six groups of five mice each received intraperitoneal (IP) injections with 0.2 mL of inoculum (Groups 2-7, Table at right). A control group of five mice received the saline-mucin solution only.

Treatment Groups

Group	Number Of Mice	Treatment
1	5	Vehicle Only
2	5	$4 \times 10^6$ CFU <i>S. aureus</i>
3	5	$2 \times 10^7$ CFU <i>S. aureus</i>
4	5	$1 \times 10^8$ CFU <i>S. aureus</i>
5	5	$5 \times 10^8$ CFU <i>S. aureus</i>
6	5	$1 \times 10^9$ CFU <i>S. aureus</i>
7	5	$2 \times 10^9$ CFU <i>S. aureus</i>

The mice were monitored for up to 7 days and the portion surviving in each group was determined (see top figure at right).

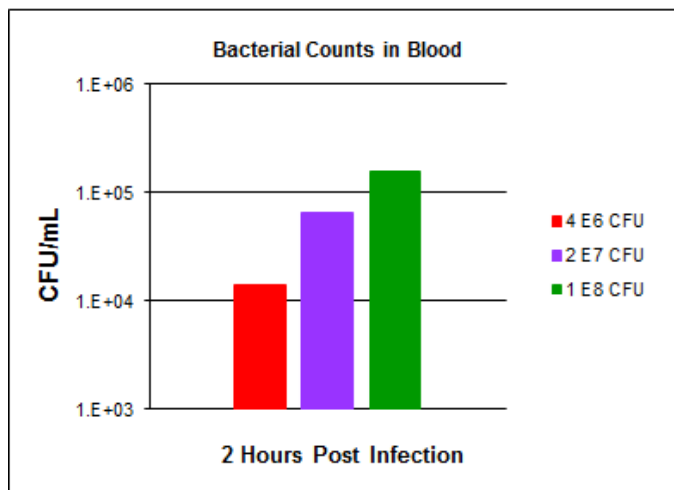
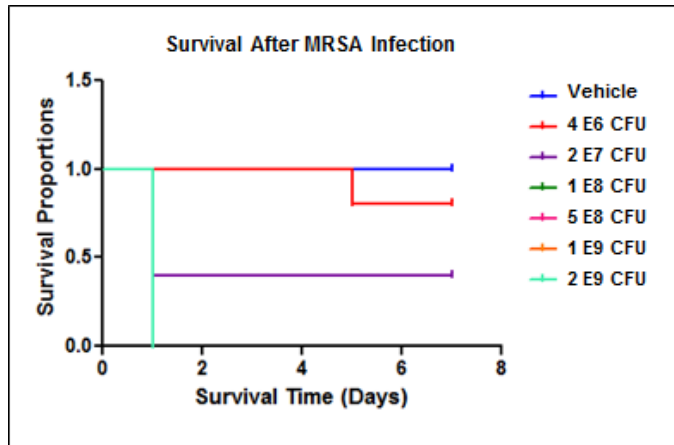
The results demonstrate that survival was dose dependent. All mice in Group 1 which received only the vehicle survived for 7 days.

100% of the mice in Group 2 (4 E6 CFU) survived for at least 5 days whereas only about 40% of the mice in Group 3 (2 E7 CFU) survived in this same time period.

The inoculum used for Groups 4, 5, 6, and 7 produced complete lethality in all mice within 24 hours

Blood samples were collected from mice at various time points post inoculation. Samples were frozen after collection and stored at  $-80^{\circ}\text{C}$  until analyzed. Subsequently, the samples were thawed at room temperature and bacterial counts determined by dilution and plating.

Results of analysis of blood samples taken at 2 hours post infection for Groups 2, 3 and 4 are shown in the bottom figure at right. The concentration of bacteria in the blood correlates with bacterial dose.



## Summary

The mouse peritonitis-sepsis model of infection is a standard method of testing antimicrobial agents. The model can also be adapted with the use of cyclophosphamide to induce neutropenia.

Two endpoints are commonly used for measuring the effect of antibiotics:

- Bacterial counts in body fluids or tissues
- Death (or survival) of the animal

The mouse peritonitis-sepsis model is an important early screening method to study *in vivo* effects of antibacterial compounds and provides a natural step in testing of antibiotics *in vivo* before moving to larger animals or humans.

## ImQuestSUCCESS

### Select drug candidates with the highest probability of clinical success

The ImQuestSUCCESS preclinical services platform is used to critically evaluate the potential of a test compound and to assure that its efficacy, toxicity, and pharmaceutical properties are evaluated in a comprehensive and interactive way. Successful completion of platform objectives provides significant confidence in the potential of a test compound to transition to human clinical trials, enhances the robustness of drug development efforts and reduces the risk of expensive clinical development failures by the exclusion of candidates which are likely to fail during advanced preclinical and clinical development at early (and less expensive) time points.