# QUANTIFICATION OF VIABLE BACTERIA FROM INFECTED MOUSE TISSUE

## RECOVERY OF VIABLE BACTERIA AFTER HOMOGENIZATION USING PRECELLYS EVOLUTION

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## CONTEXT

Clinical isolates of hypervirulent bacteria are often highly capsulated. This limits the tagging of these bacteria with fluorescent or luminescence markers, hence eliminating the use of *in vivo* live imaging to rapidly capture bacterial load in the organs of small animal models. Instead, conventional protocols requiring large sample numbers from each time-point and laborious **homogenization of tissues** are often used to quantify these bacteria that disseminate into the tissues. Here, we present the method of using **Precellys Evolution homogenizer** to speed up tissue homogenization in an efficient, safe, and consistent way to simplify bacterial quantification in tissues. This method allows high throughput processing of organs to understand bacterial dissemination into the organs for tropism and pathogenesis studies.

## MATERIALS

- **Precellys Evolution** (Bertin Technologies, Montigny-le-Bretonneux, France)
- For Livers (large organs): 7 ml tube (BER P000944-LYSK0-A.0) and 1.4 mm Zirconium oxide beads (BER P000927-LYSK0-A.0). Tubes were self-filled. *Alternative:* Pre-filled tubes CK14 7 ml (P000940-LYSK-A.0)
- For lungs, spleen, colon and feces (small organs): 2 ml tube (BER P000943-LYSK0-A.0) and 1.4 mm Zirconium oxide beads (BER P000927-LYSK0-A.0). Tubes were self-filled. *Alternative:* Pre-filled tubes CK14 2 ml (P000912-LYSK-A.0)
- LB broth + Carbenicillin (100 ng/ml)
- LB agar + Carbenicillin (100 ng/ml)
- Bacteria infected mice

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## PROTOCOL

- **Evaluation of Bacterial killing due to homogenization:**

The loss of bacterial directly due to homogenization was tested in **Precellys Evolution** 2 ml tube with 1.4 mm Zirconium oxide beads. Bacterial is grown to OD value of 1 at absorbance OD-600 reading. 1ml of bacteria is set aside as controls, while the other 1ml of bacteria is subjected to **homogenization** at 6,800 rpm, 2 x 30 sec with 1 min delay in between each cycle. Content in the controls and the homogenized tube were serial diluted and spotted to determine bacterial concentration as an indicator of bacterial killing.

- **Bacterial quantification in livers**:

Entire liver is excised from the mouse, weighed, and placed into 7 ml Precellys tube with 1.4 mm Zirconium oxide beads and 2 ml of LB broth. Tissues are homogenized at 6,800 rpm, 2 x 30 sec with 1 min delay in between each cycle. Unwanted tissue homogenate is spin down at 300G for 5 min, while the bacterium in the supernatant is collected, serial diluted and spotted onto LB agar for bacterial quantification.

- **Bacterial quantification in lungs, spleen, bladder and colon**:

Tissues are collected from the mouse, weighed, and placed into respective 2 ml **Precellys** tube with 1.4mm Zirconium oxide beads and 1 ml of LB broth. Tissues are homogenized at 6,800 rpm, 2 x 30 sec with 1 min delay in between each cycle. Unwanted tissue homogenate is spin down at 300G for 5 min, while the bacterium in the supernatant is collected, serial diluted and spotted onto LB agar for bacterial quantification.

## RESULTS

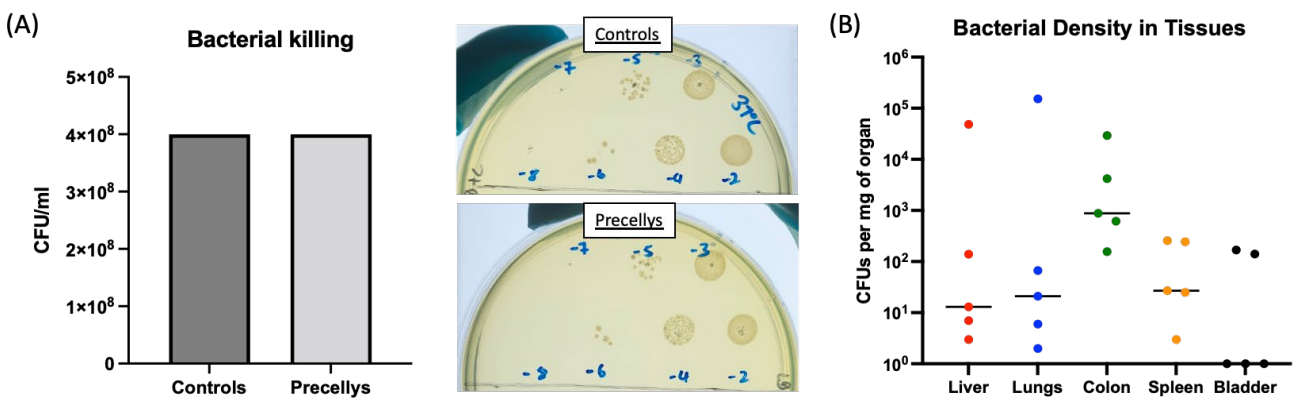


*Figure 1: Quantification of bacteria dissemination into the tissues using Precelly Evolution system. (A) Loss of bacterial titre due to precelly homogenization protocol. Titre obtained from bacterial spotting was plotted into bar chart of the left panel. (B) Dissemination and bacterial load in different soft tissues. Data were normalized to the weight of each organ.*

The **Precellys homogenization protocol** employed to mesh up soft tissues of interests in this study did not lead to any unwanted bacterial killing that could diminish the detection limit of the experimental design (Figure 1A). Importantly, despite not introducing temperature controls that was available with the Precellys Evolution system, tubes of the homogenized samples were not heated up in the cycle condition used (data not shown).

Liver, lungs, colon, spleen, and bladder were completely homogenized in the rapid cycle of 2 x 30 s, 6,800 rpm with 1 min delay. Importantly, bacteria were successfully recovered from these organs and quantified rapidly (Figure 1B) with most of the samples gave reading above detection limit.

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## CONCLUSION

The settings we used with **Precellys Evolution** system allow rapid and consistent quantification of bacterial load in soft tissues. Such system will be useful for follow-up in treatment of mice infected with clinical isolate of bacteria where fluorescent or luminescence tagging are often unavailable.

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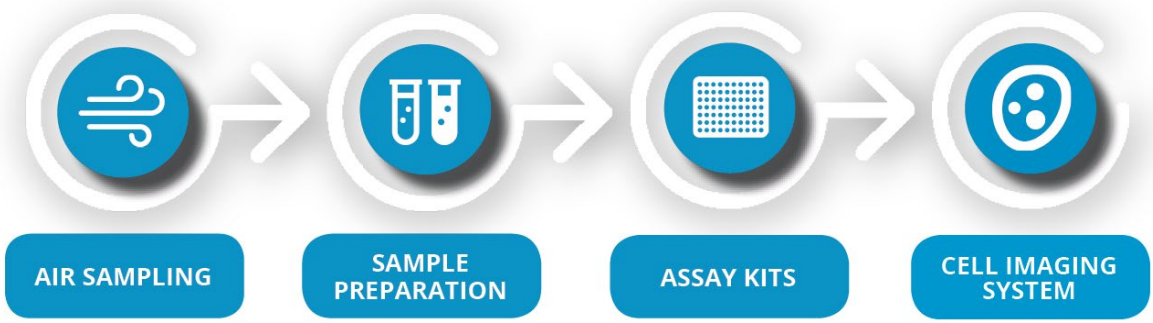


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