

Maintaining bacterial viability during tissue homogenization Illawarra Health and Medical Research Institute, University of Wollongong, Australia

CONTEXT

In an animal model of invasive infections caused by *Streptococcus pyogenes*, bacteria will colonize various tissues and organs. Bacterial loads present in these tissues is determined by homogenization, serial dilution and plating onto nutrient agar.

This study investigates the use of the Precellys 24 for tissue homogenization.

MATERIAL

- Precellys 24 homogenizer.
- Precellys kits: 03961-1-003 (1.4mm ceramic beads) and 03961-1-002 (2.8mm ceramic beads).
- Samples: skin (122.3 mg) and spleen tissues (38.6 mg).
- Bacteria culture: *S. pyogenes* was grown in Todd Hewitt Broth supplemented with 1% yeast extract at 37°C until an $A_{600} = 0.5$.
- Buffer: 0.7% sterile saline (1000µl) inoculated with *S. pyogenes* (T0 Inoculum).

PROTOCOL

Note that pathogenic *S. pyogenes* must be processed under appropriate biosafety containment.

- Precellys24 parameters:
 - Spleen: 6000 rpm, 1x30 sec,
 - Skin: 6000 rpm, 2x30 sec or 6000 rpm, 4x30 sec.
- Analysis: assessment of *S. pyogenes* concentration (CFU/ml) by cultural method.

RESULTS

Following tissue homogenization, the number of viable bacterial cells (CFU/ml) present was determined by serial dilution in sterile 0.7% saline and plating onto nutrient agar.

Results (see Figure1) indicate that for all treatment groups the type of bead and the processing time had no significant effect on the viability of *S. pyogenes* (t test $p > 0.1$).

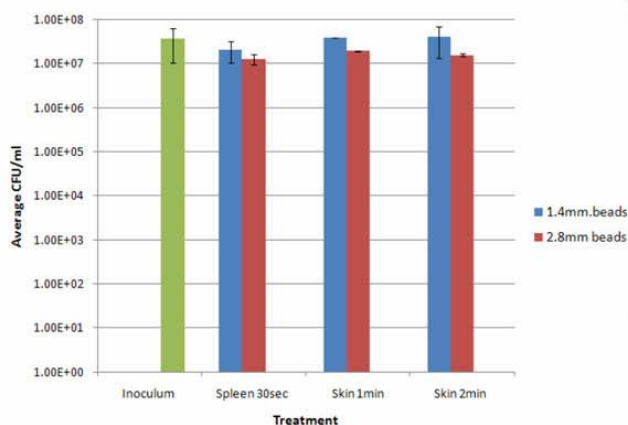
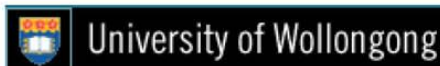


Figure 1: The effect of tissue homogenization on the viability of *S. pyogenes*. Data represents the mean CFU/ml from triplicate samples with error bars indicating the standard deviation. The inoculum represents the CFU/ml added to each sample.



CONCLUSION

Tissue homogenization (spleen & skin) using the Precellys 24 with ceramics beads kits (1.4mm or 2.8mm) does not affect the viability of the Gram positive pathogen *S. pyogenes* present in these samples. The bacterial viability is maintained during tissue homogenization.

The potential to process up to 24 individual samples in a short time period with no risk of cross-contamination has considerable benefits over traditional homogenization methods.

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