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Customer protocol

**Determination of parasite load from skin tissue infected with *Leishmania major***

**Methods**

1. Prepare collagenase medium freshly.
2. Split mouse ear into halves along the cartilage.
3. Add 1.5 mL collagenase medium per 6-well and incubate one mouse ear for 2 hours at 37 °C, 5% CO₂.
4. Transfer both halves of one ear into a gentleMACS C Tube.
5. Tightly close the C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
6. Run the defined gentleMACS Program (refer to figure 1 and table 1). For more information about creating new programs, refer to the gentleMACS Dissociator user manual.
7. After termination of the program, detach C Tube from the gentleMACS Dissociator.
8. Discard cap of C Tube and replace it by a cap of a gentleMACS M Tube and run gentleMACS Program B.
9. Apply cell suspension to a cell strainer (70 µm mesh size) placed on a 50 mL tube.
10. Wash the cell strainer twice with 5 mL PBS.
11. Discard cell strainer and centrifuge sample at 3000xg for 8 minutes.
12. Resuspend cell pellet in 1 mL drosophila medium.
13. Serially dilute 100 µL sample to determine parasite load.

**Figure 1:** User-defined gentleMACS Program.

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**Beate Lorenz and Esther von Stebut-Borschitz***
Department of Dermatology, Johannes-Gutenberg University, Mainz, Germany
*Corresponding author (vonstebu@mail.uni-mainz.de)

**Background**

In cutaneous *Leishmania major* (*L. major*) infections of mice, the existence of Th1/Th2 cells has been identified more than 20 years ago. Nowadays, the role of additional T cell populations as well as B cell-mediated immunity are well established. Granuloma formation in mice is dependent on the ability to efficiently mount anti-*Leishmania* immunity. If this process fails, early parasite dissemination into, e.g., visceral organs, occurs.

This protocol describes a model for determining lesional parasite loads after experimental *L. major* infection for a correlation to lesion sizes at the inoculation site. Both the size of the lesion as well as information about parasite containment versus spreading to visceral organs are important parameters for determining disease outcome in mice.

**Materials and methods**

**Materials**

- gentleMACS Dissociator™ or gentleMACS Octo Dissociator
- gentleMACS C Tubes and M Tubes
- Incubator (37 °C, 5% CO₂)
- 6-well cell culture plate
- Cell strainer (70 µm mesh size)
- Collagenase medium (100 mg collagenase and 1 mL Penicillin and Streptomycin per 100 mL DMEM medium)
- Drosophila medium
- Phosphate-buffered saline (PBS)
Results

The gentleMACS Octo Dissociator provides identical high quality results of parasitic loads compared to a competitive instrument used for automated mechanical tissue disaggregation, which was well established in our lab. In addition to high quality results the gentleMACS Octo Dissociator is an easy-to-use system that provides users with the advantages of increased user safety and significant reduction of labor time.

Table 1: Defines the time given in seconds (sec) required to reach the needed speed. This is given in revolutions per minute (rpm).

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</table>

Figure 2: BALB/c and C57BL/6 mice were infected with L. major (2×10⁵ parasites per ear). Parasite load was determined 3 and 6 weeks later.

Figure 3: Comparison of labor time: 8 mouse ears were prepared with the gentleMACS Octo Dissociator and a competitive instrument in parallel. Using the gentleMACS Octo Dissociator saves about 1 hour of working time compared to an instrument frequently used to deliver automated mechanical tissue disaggregation.

Conclusion

L. major parasite load analysis can be accomplished with ease, saving of time, and high reproducibility using a robust experimental set up based on the gentleMACS Octo Dissociator.

References


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