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| **ARRIVE Essential 10** | |
| **Study Design** | A total of **five male C57BL/6 mice (7 weeks old, Charles River Laboratories)** were used in this study. **Three mice** were used to collect parasites for single-cell RNA sequencing (scRNA-seq), while **two additional mice** were used for microscopy analysis on **day 5 post-infection (p.i.)**. All mice were housed in the same cage and were siblings. |
| **Sample size** | Each mouse was infected intraperitoneally at **7 weeks of age** with **2000 *T. brucei* parasites**. **Three mice** were used for parasite collection at **day 6 p.i.**, ensuring sufficient biological heterogeneity and enough parasites for four samples. **Two additional mice** were sacrificed at **day 5 p.i.** for microscopy analysis of parasite motility before encapsulation. |
| **Inclusion and Exclusion Criteria** | Only animals that presented detectable **parasitemia at days 5-6 p.i.** were included for parasite collection. All **five infected mice developed parasites in the blood and were used for the experiment**. No animals were excluded. |
| **Randomization** | No control group was included, as the objective of the study was **to collect parasites from infected animals at specific time points (days 5 and 6 p.i.)**. Therefore, randomization was not applicable. |
| **Blinding** | Blinding was not performed, as **we were aware of which animals were infected**. |
| **Outcome Measures** | **Parasitemia was measured on days 4, 5, and 6 p.i.** to confirm infection.  **Mice were monitored daily for health status and distress signs.**  **Microscopy analysis** was performed on **day 5 p.i.** on two mice to observe parasite motility before encapsulation.  **Parasite collection for scRNA-seq** was performed on **day 6 p.i.** from three mice. |
| **Statistical Methods** | No statistical analysis was performed. As this study focused solely on parasite collection and not comparative analysis, no statistical tests were required. |
| **Experimental Animals** | A total of **five male C57BL/6 mice (7 weeks old, Charles River Laboratories)** were used. All mice were **siblings, housed together, and had no genetic modifications or prior experimental procedures**. |
| **Experimental Procedures** | Mice were infected with **2,000 *T. brucei* parasites** in **HMI-11 medium**, delivered in a **200 µL volume via intraperitoneal injection** between **4:00–5:00 PM** using a **1 mL insulin syringe**. Mice were monitored **two hours post-infection and daily thereafter**.   * **On days 4, 5, and 6 p.i.**, **1 µL of blood** was collected from the **tail vein** to assess **parasitemia**. * **On day 5 p.i., two mice were sacrificed, and parasites were analyzed by microscopy** to observe motility before encapsulation. * **On day 6 p.i., three mice were sacrificed for parasite collection for scRNA-seq.**   Mice were euthanized using a **pre-calibrated CO₂ chamber approved by the animal facility**. The **CO₂ flow rate and exposure time were automatically controlled** following institutional guidelines. Mice were first **anesthetized with CO₂ before euthanasia**, and **death was confirmed visually by the absence of respiratory movement** before proceeding with **parasite collection**. |
| **Results** | All five mice developed detectable parasitemia and were successfully used for either microscopy (day 5 p.i.) or parasite collection (day 6 p.i.). |

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| **ARRIVE Recommended Set** | |
| **Abstract** | This study investigates how *Trypanosoma brucei* motility influences microfluidics capturing for single-cell RNA sequencing (scRNA-seq). Using the 10x Genomics platform, we analyzed the impact of temperature on parasite retention, showing that rapid cooling to 0°C prior to microfluidics capturing significantly improves recovery of highly motile slender forms relative to room temperature conditions. We infected three male C57BL/6 mice (7 weeks old, Charles River Laboratories) with 2,000 parasites, and parasites were isolated from blood on day 6 post-infection (p.i.). Our findings emphasize the impact of motility in microfluidic-based single-cell methods and suggest protocol optimizations to reduce bias in scRNA-seq datasets. |
| **Background** | scRNA-seq has revolutionized transcriptomic studies by enabling single-cell resolution analysis of gene expression. However, most microfluidic systems, including 10x Genomics, were designed for non-motile cells. *T. brucei* is a highly motile flagellated parasite whose slender forms exhibit speeds up to 20-50 µm/s, potentially affecting encapsulation efficiency. Here, we investigated whether reducing motility via rapid cooling improves parasite encapsulation and scRNA-seq data quality. |
| **Objectives** | The objective of this study was to evaluate whether *T. brucei* motility influences its encapsulation efficiency in the 10x Genomics system and to test whether rapid cooling to 0°C before encapsulation improves parasite recovery. |
| **Ethical statement** | All procedures involving animals were approved by the "Órgão Responsável pelo Bem-estar Animal" (ORBEA) of Instituto de Medicina Molecular and the "Direcção Geral de Alimentação e Veterinária", under licenses 018889/2016 and 017549/2021, in compliance with EU regulations for animal experimentation. |
| **Housing and husbandry** | Mice were group-housed in specific-pathogen-free (SPF) conditions in filter-top cages. They were maintained at 21–22°C, 45–65% humidity, with a 12-hour light/dark cycle, and had ad libitum access to food and water. |
| **Animal care and monitoring** | Mice were monitored daily for signs of distress, including lethargy, weight loss, and abnormal behavior. To minimize stress, blood collection was performed via tail vein puncture, and euthanasia was conducted humanely using a CO₂ chamber. No unexpected adverse events occurred. Humane endpoints (e.g., weight loss >20%, severe lethargy) were established but not reached in any animal. |
| **Interpretation/scientific implications** | Our results demonstrate that parasite motility reduces encapsulation efficiency in microfluidic-based single-cell approaches, leading to a biased loss of highly motile slender forms. Rapid cooling before encapsulation reduces this bias and improves recovery without altering transcriptomic profiles. |
| **Generalisability/translation** | While this study focuses on *T. brucei*, the findings are relevant for other highly motile unicellular organisms that undergo scRNA-seq, such as Leishmania spp., *T. cruzi*, or motile bacteria. |
| **Protocol registration** | No formal protocol registration was performed. |
| **Data access** | Raw sequencing files are available in and analysis scripts, are available at <https://www.ncbi.nlm.nih.gov/sra/PRJNA1227990> under the project name “Cell motility influences the encapsulation process in scRNAseq microfluidics” under the reference PRJNA1227990. All bioinformatic codes are available in GitHub https://github.com/Laritabonita/Encapsulation |
| **Declaration of interests** | The authors declare no competing interests. |