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Data Article

# Dataset on *in vitro* maintenance of *Mansonella perstans* microfilariae and drug testing



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# ABSTRACT

Endemic communities of *Mansonella perstans* infections have been neglected since associated pathology remains undefined. Consequently, improvements in drug therapy have also been ignored despite a large number of infected individuals in areas of Cameroon. Thus, we established an *in vitro* system to culture *M. perstans* microfilariae (Mf); the transmission stage of infection. In short, we compared the ability of two renowned culture media (Dulbecco's Modified Eagle's Medium (DMEM) and Roswell Park Memorial Institute (RPMI-1640)) to sustain Mf in culture. Media were supplemented with 10% fetal bovine serum (FBS) and monkey kidney epithelial cells (LLC-MK<sub>2</sub>) were used as feeder cells. As readout we assessed Mf survival and motility using a standardised

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microscopy assessment strategy. Moreover, this *in vitro* culture system was used to test susceptibility levels of microfilariae to different chemotherapeutic agents. Parasite motility was scored daily using a graded system and analysed using the average motility and area under the motility curve of *M. perstans* Mf. These datasets were analysed and discussed in detail in the related article entitled: *"In vitro* maintenance of *Mansonella perstans* microfilariae and its relevance for drug screening" [1].

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Specifications Table

Subject Specific subject area	Parasitology In vitro culture systems
Type of data	Table
How data were acquired	Microscopy
Data format	Raw and analysed
Parameters for data collection	<i>In vitro</i> cultures were performed by culturing 30–50 microfilariae/well in a 48-well flat-bottomed plate with or without a confluent monolayer of monkey kidney epithelial cells (LLC-MK <sub>2</sub> ) as feeder cells in either RPMI-1640 or DMEM media supplemented with 5 $\mu$ g/ml ciprofloxacin and 10 $\mu$ g/ml fluconazole in the presence or absence of 10% fetal bovine serum (FBS).
Description of data collection	Microfilariae motility was scored daily using microscopy (x10 magnification) by applying a 4-point grading scale: 0, no movement or immotile; 1, intermittent shaking of head and tail; 2, sluggish (shaking of the whole form whilst the microfilariae remain stationary); 3, vigorous movement (shaking of the whole form with migration around the well).
Data accessibility	Within the article
Related research article	Njouendou AJ, Kien CA, Esum ME, Ritter M, Chounna Ndongmo WP, Fombad FF, Gandjui NVT, Njiokou F, Enyong P, Pfarr K, Turner J, Layland LE, Hoerauf A, Wanji S. " <i>In vitro</i> maintenance of <i>Mansonella perstans</i> microfilariae and its relevance for drug screening." Experimental Parasitology 2019; DOI: https://doi.org/10.1016/j.exppara.2019.107769

#### Value of the Data

- Scoring of microfilariae motility will be useful for the assessment of suitable conditions for filarial survival as well as testing drug efficacy.
- Data presented here can be used as reference for further culture of *M. perstans* Mf.
- Data processing approaches of these datasets is easy to replicate and relevant for the comparative analysis of the motility of filarial species in vitro.
- The dataset supplied with this article can be subsequently used for meta-analysis.
- The described analytical approach will be useful to assess the efficacy of chemotherapeutic agents against Mf.

## 1. Data

The findings presented here are based on the previous publication entilted "*In vitro* maintenance of *Mansonella perstans* microfilariae and its relevance for drug screening" [1]. Data on the optimization of the *in vitro* culture conditions for the maintenance of *M. perstans* Mf are summarized in Table 1 (RPMI-1640) and Table 2 (DMEM), showing the daily average motility and areas under the curve (AUC) of *M. perstans* Mf activity.

#### Table 1

Average motility and area under the curve (AUC) of *M. perstans* Mf activity during *in vitro* cultures containing RPMI-1640 medium supplemented with or without 10% fetal bovine serum (FBS) and/or monkey kidney epithelial feeder cells (LLC-MK<sub>2</sub>) over 20 days.

Incubation days	Cell-free		LLC-MK <sub>2</sub>	
	Serum-free	10% FBS	Serum-free	10% FBS
0	100	100	100	100
1	99.58	99.3	100	99.78
2	81.02	98.29	100	100
3	72.25	95.76	100	100
4	41.86	84.84	100	100
5	28.46	82.31	100	100
6	26.5	80.68	100	100
7	25.36	79.1	100	100
8	25.36	79.1	100	100
9	25.24	79.1	100	100
10	25.24	79.1	100	100
11	25.24	79.1	100	100
12	25.24	79.1	100	100
13	25.24	79.1	100	99.63
14	25.24	79.1	100	100
15	25.24	79.1	100	99.62
16	25.24	79.1	100	99.63
17	25.24	79.1	100	99.46
18	25.24	79.1	100	99.46
19	25.38	78.73	100	99.46
20	25.38	78.73	100	99.46
Average AUC (%)	37.04	82.92	100	99.84
<i>p</i> -value <sup>a</sup>	-	0.2623	0.0003	0.0034

Abbreviations. AUC: area under the curve.

<sup>a</sup> Pairwise comparisons using Dunn's-test for multiple comparisons of independent samples. *p*-values presented in the table compare serum free and 10% FBS in the presence or absence of feeder cells (LLC MK<sub>2</sub> cells).

#### 2. Experimental design, materials, and methods

*M. perstans* Mf extracted from human blood were cultured *in vitro* as recently described for *Loa loa* parasites [2–4]. Briefly, 30–50 Mf/well were cultured in a 48-well flat-bottomed plate (Corning, New York, USA) with or without confluent monolayers of LLC-MK<sub>2</sub> (LGC Standard GmbH, Wesel, Germany) as feeder cells in either RPMI-1640 or DMEM medium (Gibco, Cergy-Pontoise, France) supplemented with 5  $\mu$ g/ml ciprofloxacin and 10  $\mu$ g/ml fluconazole (Sigma Aldrich, St Louis, MO, USA) and in the presence or absence of 10% FBS (Lonza, Verviers, Belgium). Cultures were incubated at 37 °C and 5% CO<sub>2</sub> for 20 days and helminth viability was evaluated by grading motility overtime. Mf motility was scored on a daily basis in a blind manner using x10 magnification with an inverted microscope (Motic, Wetzlar, Germany) by applying a 4-point scale:

- 0, no movement or immotile;
- 1, intermittent shaking of head and tail;
- 2, sluggish (shaking of the whole form whilst the Mf remain stationary);
- 3, vigorous movement (shaking of the whole form with migration around the well).

Raw data were saved in a spreadsheet and using the above described 4-point grading scale the percentage (%) of motility was calculated according to the following formula:

Motility (%) = 
$$\frac{\sum SiNi}{3. \sum Ni} \times 100$$

where Si is the score of point scale i and Ni is the total number of worms at a point scale I [5].

#### Table 2

Average motility and area under the curve (AUC) of *M. perstans* Mf activity during *in vitro* cultures containing DMEM medium supplemented with or without 10% fetal bovine serum (FBS) and/or monkey kidney epithelial feeder cells (LLC-MK<sub>2</sub>) over 20 days.

Incubation days	Cell-free		LLC-MK <sub>2</sub>	
	Serum-free	10% FBS	Serum-free	10% FBS
0	100	100	100	100
1	99.2	99.49	100	100
2	99.3	98.83	100	99.76
3	88.97	95.17	100	99.76
4	80.04	87.43	100	99.76
5	77.55	84.41	100	99.76
6	77.8	79.37	100	99.76
7	77.64	79.26	100	99.76
8	77.52	79.26	100	99.76
9	77.71	79.26	100	99.76
10	77.71	78.99	100	99.76
11	77.71	79.26	100	99.76
12	77.71	79.26	100	99.76
13	77.71	79.26	100	99.42
14	77.71	79.26	99.72	99.42
15	77.71	79.26	100	99.42
16	77.71	79.26	100	99.42
17	77.71	79.26	100	99.42
18	77.71	79.26	100	99.42
19	77.71	79.13	100	99.42
20	77.71	79.13	100	99.42
Average AUC (%)	81.08	83.21	99.99	99.65
p-value <sup>a</sup>	-	0.999	0.0041	0.0110

Abbreviations. AUC: area under the curve.

<sup>a</sup> Pairwise comparisons using Dunn's-test for multiple comparisons of independent samples. *p*-values presented in the table compare each system to the serum free and cell free system.

The mean of the area under curve (AUC) was calculated using the percentage motility of the drugs from 0 to 5 days according to the following formula:

AUC = 
$$\frac{\sum_{i=0}^{n-1} (t_{i+1} - t_i)(y_{i+1} + y_i)}{t_{n-1}}$$

where AUC = area under curve; y = motility; t = time point; n = an integer [6].

The effects of media and supplements on the Mf motility was compared using non-parametric tests. The Kruskal-Wallis one-way analysis test was used to assess the global significant differences between the median AUC and Dunn's post-hoc test was applied for pairwise multiple comparisons of the ranked data. This analysis was performed using the Pairwise Multiple Comparisons of Mean Rank Sums (*PMCMR*) package in R version 3.4.1 [7].

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### **Conflict of Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

# Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2019.104930.

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