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

Abdel Jelil Njouendou a, b, 1, Manuel Ritter c, 1, Chi Anizette Kien a, d, Mathias E. Esum a, d, Winston Patrick Chounna Ndongmo a, d, Fanny Fri Fombad a, e, Narcisse Victor T. Gandjui a, d, Flobert Njiokou f, Peter Enyong a, d, Kenneth Pfarr c, g, Joseph Turner h, Laura E. Layland c, g, Achim Hoerauf c, g, Samuel Wanji a, d, *

a Research Foundation for Tropical Diseases and the Environment (REFOTDE), Buea, Cameroon
b Department of Biomedical Sciences, Faculty of Health Sciences, University of Buea, Buea, Cameroon
c Institute of Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Germany
d Parasite and Vector Research Unit (PAVRU), Department of Microbiology and Parasitology, University of Buea, Buea, Cameroon
e Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, Buea, Cameroon
f Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde I, Yaounde, Cameroon
g German Center for Infection Research (DZIF), Bonn - Cologne Partner Site, Bonn, Germany
h Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, UK

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-MK2) were used as feeder cells. As readout we assessed Mf survival and motility using a standardised...
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].

© 2019 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

### Specifications Table

| Subject                     | Parasitology                      |
|-----------------------------|-----------------------------------|
| Specific subject area       | In vitro culture systems          |
| Type of data                | Table                             |
| How data were acquired      | Microscopy                        |
| Data format                 | Raw and analysed                  |
| Parameters for data collection | in vitro 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-MK2) as feeder cells in either RPMI-1640 or DMEM media supplemented with 5 μg/ml ciprofloxacin and 10 μg/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, Njikou F, Enyong P, Pfarr K, Turner J, Layland LE, Hoerauf A, Wanji S. “In vitro maintenance of Mansonella perstans 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 entitled “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.
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) without or with confluent monolayers of LLC-MK2 (LGC Standard GmbH, Wesel, Germany) as feeder cells in either RPMI-1640 or DMEM medium (Gibco, Cergy-Pontoise, France) supplemented with 5 μg/ml ciprofloxacin and 10 μg/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% CO2 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:

\[
\text{Motility (\%)} = \frac{\sum S_i N_i}{3 \cdot \sum N_i} \times 100
\]

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

| Incubation days | Cell-free | LLC-MK2 |
|-----------------|-----------|---------|
|                 | 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 (%)**

|                | Cell-free | LLC-MK2 |
|----------------|-----------|---------|
| Serum-free     | 37.04     | 82.92   |
| 10% FBS        | 25.24     | 78.73   |

*p*-value\(^a\)

|                | Cell-free | LLC-MK2 |
|----------------|-----------|---------|
| Serum-free     | 0.6223    | 0.0003  |
| 10% FBS        | 0.0034    | 0.0034  |

Abbreviations. AUC: area under the curve.

\(^a\) 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 MK2 cells).
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:

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

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].

**Acknowledgments**

We sincerely thank the Mf-positive individuals for donating their blood in this study. In addition, we thank the German Research Foundation (DFG; Grant DFG HO2009/10-1; HO 2009/14-1) and the Federal Ministry of Education and Research (BMBF initiative Research Networks for Health Innovations in Sub-Sahara Africa: TAKeOFF) for financial support.

**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.

---

**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-MK2) over 20 days.

| Incubation days | Cell-free |  | LLC-MK2 |  |
|-----------------|-----------|----------------|---------|----------------|---------|
|                 | 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      | 99.76           |
| 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*: 0.999 | 0.0041 | 0.0110 |

Abbreviations. AUC: area under the curve.

* 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.
Appendix A. Supplementary data

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

References

[1] A.J. Njouendou, C.A. Kien, M.E. Esum, M. Ritter, W.P. Chounna Ndongmo, F.F. Fombad, et al., *In vitro* maintenance of *Mansonella perstans* microfilariae and its relevance for drug screening, Exp. Parasitol. 206 (2019) 107769.
[2] D. Zofou, F.F. Fombad, N.V.T. Gandjui, A.J. Njouendou, A.J. Kengne-Ouafo, P.W. Chounna Ndongmo, et al., Evaluation of *in vitro* culture systems for the maintenance of microfilariae and infective larvae of *Loa loa*, Parasites Vectors 11 (1) (2018) 275.
[3] A.J. Njouendou, F.F. Fombad, M. O’Neill, D. Zofou, C. Nutting, P.C. Ndongmo, et al., Heterogeneity in the *in vitro* susceptibility of *Loa loa* microfilariae to drugs commonly used in parasitological infections, Parasites Vectors 11 (1) (2018) 223.
[4] A.J. Njouendou, M. Ritter, W.P.C. Ndongmo, C.A. Kien, G.T.V. Narcisse, F.F. Fombad, et al., Successful long-term maintenance of *Mansonella perstans* in an *in vitro* culture system, Parasites Vectors 10 (1) (2017) 563.
[5] F.F. Fombad, A.J. Njouendou, P.C. Ndongmo, M. Ritter, V.C. Chunda, H.M. Metuge, et al., Effect of flubendazole on developing stages of *Loa loa* in *vitro* and in *vivo*: a new approach for screening filaricidal agents, Parasites Vectors 12 (1) (2019) 14.
[6] J. Peacock, J.L. Peacock, P. Peacock, Oxford Handbook of Medical Statistics, Oxford University Press, 2011.
[7] R Core Team, A Language and Environment for Statistical Computing, 3.4.1 ed., Foundation for Statistical Computing, Vienna, Austria, 2014.