**Article Title**

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

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**Abstract**

The data presented here were collected from *in vitro* culture of *M. perstans* microfilariae, while asssessing the ability of two basic culture media; Dulbecco’s Modified Eagle’s Medium (DMEM) and Roswell Park Memorial Institute (RPMI-1640) supplemented with 10% fetal bovine serum (FBS) and a monkey kidney epithelial cell line (LLC-MK2) to support the survival of this parasite stage; and their sussceptibility to chemotherapeutic agents. Parasites motlity were scored daily on a 4 scales system, and the average motility computed, as well as the area under the motility curve. Mortility was determined as the percentges of imotiled worms.

**Keywords**

Motility, microfilaria*, in vitro, Mansonella perstans*

**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 of *M. perstans* microfilariae were performed by culturing 30 – 50 mf/well in a 48-well flat-bottomed plates with or without confluent monolayers of monkey kidney epithelial cell line (LLC-MK2) as feeder cells in either RPMI-1640 or DMEM media supplemented with 5 μg/ml ciprofloxacin and 10 μg/ml fluconazole and either with or without 10 % fetal bovine serum (FBS). |
| **Description of data collection** | Microfilaria motility was daily scored under x10 magnification using an inverted microscope 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 remained stationary); 3, vigorous movement (shaking of the whole form and with migration around the well). |
| **Data accessibility** | Data is provided in this article |

**Value of the Data**

* Scoring of the microfilariae motility is useful for the assessment of suitable conditions for parasites survival as well as testing drug efficacy.
* Data presented here can be used as reference for further culture of *M. perstans* microfilariae.
* Data processing approach of this 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.
* Analytical approach here can be useful while assessing the efficacy of chemotherapeutic agents against microfilariae.

**Data**

## Data on the optimization of the *in vitro* culture conditions for the maintenance of *M. perstans* microfilaria are summarized in table 1 for culture in RPMI and table 2 for DMEM, showing the average motility recorded daily for each culture system, the areas under the curves (AUC) and statistical analysis. The raw dataset is found in the Supplementary dataset.

**Table 1**

Average motility and area under the curve (AUC) of *M. perstans* microfilariae *in vitro* in RPMI medium supplemented with or without 10 % fetal bovine serum (FBS) and / or monkey kidney epithelial feeder cells (LLC-MK2) within 20 days.

|  |  |  |
| --- | --- | --- |
| **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 (%)** | 37.04 | 82.92 | 100 | 99.84 |
| *p.* value\* | - | 0.2623 | 0.0003 | 0.0034 |

Kruskal-Wallis rank sum test: *X2* = 21.08, *df* = 3, *p* = 0.0001013

\*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.

# Statistical differences were found between 10% FBS, Cell-free and Serum-free, LLC-MK2 systems with *p*-values of 0.0330 and 0.0116 for RPMI and DMEM respectively. However, no difference was observed between the two systems using LLC-MK2.

Abbreviations. AUC: area under the curve.

**Table 2**

Average motility and area under the curve (AUC) of *M. perstans* microfilariae *in vitro* in DMEM medium supplemented with or without 10 % fetal bovine serum (FBS) and / or monkey kidney epithelial feeder cells (LLC-MK2) within 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 | 99.42 |
| 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\* | - | 0.999 | 0.0041 | 0.0110 |

Kruskal-Wallis rank sum test: *X2*=18.52, *df* = 3, *p* = 0.0003436.

\*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.

# Statistical differences were found between 10% FBS, Cell-free and Serum-free, LLC-MK2 systems with *p*-values of 0.0330 and 0.0116 for RPMI and DMEM respectively. However, no difference was observed between the two systems using LLC-MK2.

Abbreviations. AUC: area under the curve.

**Experimental Design, Materials, and Methods**

## *M. perstans* mf extracted from human blood were cultured *in vitro* as recently described for *L. loa* parasites [1,2]. Briefly, 30 – 50 mf/well were cultured in a 48-well flat-bottomed plates (Corning, New York, USA) with or without confluent monolayers of LLC-MK2 (LGC Standard GmbH, Wesel, Germany) as feeder cells in either RPMI-1640 or DMEM media (Gibco) supplemented with 5 μg/ml ciprofloxacin and 10 μg/ml fluconazole (Sigma Aldrich) and either with or without 10 % FBS (Lonza, Verviers, Belgium). Cultures were incubated at 37 °C and 5 % CO2 for 20 days and parasite viability was evaluated using their motility as the primary indicator. Mf motility was daily scored under x10 magnification using 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 remained stationary);

## - 3, vigorous movement (shaking of the whole form and with migration around the well).

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

$$ Motility \left(\%\right)=\frac{\sum\_{}^{}SiNi}{3.\sum\_{}^{}Ni}×100$$

where Si is the score of point scale i and Ni is the total number of worms at a point scale I [2].

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

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where AUC = area under curve; y = motility; t = time point; n = an integer [4].

The effects of media and supplements on the mf motility were 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 when a difference was detected, 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 [5].

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**References**

1. 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*, Parasit Vectors 11(1) (2018) 275.

2. 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, Parasit. Vectors 11(1) (2018) 223.

3. 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, Parasit. Vectors 12(1) (2019) 14.

4. J. Peacock, J. L. Peacock, P. Peacock. Oxford handbook of medical statistics, Oxford University Press (2011).

5. R Core Team, A Language and Environment for Statistical Computing. 3.4.1 ed. Vienna, Austria: Foundation for Statistical Computing (2014).