

# Direct Microscopy: An Alternative Tool For Assessment Of Viability Of Microfilariae

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## Abstract

Filarial infections with high morbidity still pose a challenge and consequently recognized as a major infectious disease by WHO (1) Hence, development of new drugs with synthetic or natural compounds against microfilariae becomes highly essential. Pharmacological screening of drug candidates needs to be tested for in vitro efficacy against the target organism. This eventually demands reliable, technically simple and economic method.

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## INTRODUCTION

MTT reduction assay is popular for assessing the viability of cells. This assay system depends on the chromogenic formazan product from MTT reduction by mitochondrial reducing enzyme of a viable cell (2). However the sensitivity of spectrophotometric estimation of the chromogen might be a serious limiting factor demanding for higher number of available cells. Most important technical hindrance encountered by the researchers working with common filarial worms (namely *B.malayi* and *W. bancrofti*) is the paucity of the parasite material. This problem is due to relatively rare animal model as well as the size and the number of parasites available. Hence many researchers have compromised by using direct microscopy (3) to visualize the motility rather than the actual mortality of the organism. Certain workers have to confine their research using *Setaria digitata* as the alternate model for filarial study (4), which may not be suitable to extrapolate into human filariasis model. Although technically simple and economical but the microscopy based determination is mainly criticized for the unreliability as the result may not reflect actual viability status. Hence it is almost imperative to validate the microscopy based research results against MTT assay. With this perspective present study was designed to carry out comparative evaluation of the microscopic observation against MTT assay.

## RESULTS

Approximately 15,000 microfilariae in 10 ml of RPMI media in triplicates were taken in 11 different test tubes. The first tube was kept undisturbed whereas from other ten tubes variable amount of microfilariae were taken out (ranging from 10% - 100% of the total number of parasites) and those were then killed by heating in boiling water bath for 30 min (5). The heat killed microfilariae were again added back to the respective tubes from which they were separated. First tube contained 100% live microfilariae and on the other hand the last tube contained all dead microfilariae. All of these tubes in triplicate were examined by microscopy as well as MTT assay (2).

Thus 11 samples in triplicates (ranging from 0 to 100 of live microfilariae) were analyzed and the values were tested for reproducibility. The mean percentage of immotile parasites recorded by microscopic examination was correlated with the corresponding viability values obtained through MTT assay. A very significant correlation coefficient ( $r = 0.974335$ ) was obtained suggesting that Microscopic examination can be used as an effective and reliable diagnostic tool for the study of parasite viability status. (Fig. 1) To test the robustness of the microscopic method against MTT assay we also compared the values obtained by these two methods after in vitro treatment of the parasites with various concentrations  $H_2O_2$  and observed that even in actual experimental setup the results were quite concordant.

## CONCLUSIONS

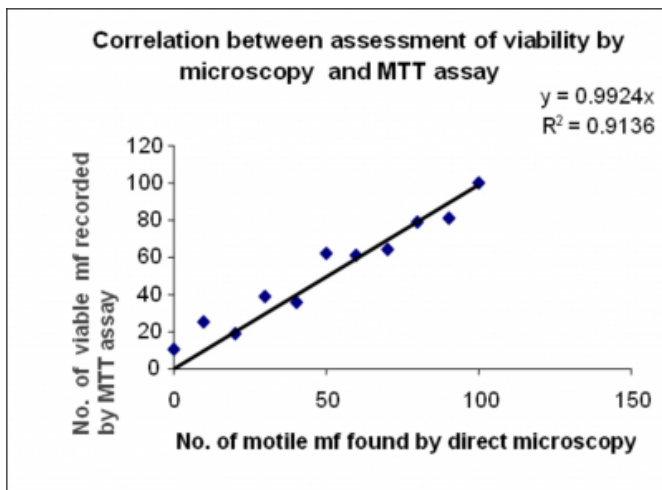
Certain previous studies on screening of some anti-filarial

agents also tried microscopy and reported corroborative results with the corresponding MTT assay values (6). However, the present study demonstrated direct evidence of significant correlation between these two assays and thus provides scientific validation of this apparently simple and economic method.

Hence we advocate that microscopic examination may be used with equal reliability as a tool for assessment of the viability of the microfilariae, which will prove immensely beneficial for the researchers working with human lymphatic filarial model.

**Figure 1**

Figure 1: Correlation between assessment of viability by microscopy and by MTT assay for mf [Each data point represent the values obtained by these two method for different amount of live parasites (0-100%)]



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