

## Modified Ritchie's method provides greater recovery of cysts of *Giardia muris*

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The Ritchie method is considered the gold standard in the detection of parasites, especially in relation to protozoa, including *Giardia muris*, the main pathogen in rodent maintained in the research laboratories. This method concentrates cysts, removes fecal debris and facilitates identification. The conventional or centrifugal sedimentation method uses ethyl ether and formaldehyde, but recent studies have described modifications aiming to decrease the toxicity and increase the efficiency, especially when molecular approaches are involved. In this context, the conventional and modified Ritchie method was tested for the detection and recovery of *Giardia muris* cysts obtained from naturally infected mice. One gram of stool from male Swiss mice with approximately 60 days old was obtained for the processing of the conventional method and each of its variables. The experiment was performed in triplicate and the data analyzed by *Software Statistica* 8.0 with significance level of 5%. The groups were divided according to the variation of the method replacing the formaldehyde by: 1) Water Milli-Q; 2) Tap water; 3) Distilled water; 4) Deionized water. A final group (5) was analyzed by the conventional method (formalin). For each variable the cysts were quantified in Neubauer's chamber and the results computed in number of cysts / gram of faeces. This study was approved by the Ethics Committee on the Use of Animals in Experimentation at the State University of Maringa under protocol number 9375170816. The highest number of cysts was observed in the group 1 ( $1.6 \times 10^5$ ), followed by 5 ( $1.2 \times 10^5$ ), 2 ( $4.0 \times 10^4$ ), 3 ( $2.6 \times 10^4$ ), 4 ( $1.2 \times 10^4$ ). Significant difference was observed between groups 1 and 3 ( $p = 0.03$ ) and between 4 and 5 ( $p = 0.02$ ). It is concluded that the change in reagents of the Ritchie method increases the amount of cysts recovered. The quality and purity of the reagents is an important factor to obtain a higher number of cysts, since the best performance was with the Milli-Q water, being this one of choice mainly when molecular approaches are involved.