

## **Influence of Ritchie method reagents on the identification and quantification of DNA from the *Giardia muris* cysts**

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Parasitological methods of concentration of cysts, such as Ritchie method, are considered gold standard in laboratory routine, as they concentrate cysts, remove fecal debris and facilitate the identification of protozoan cysts, including *Giardia muris*. The conventional Ritchie method uses ethyl ether and formaldehyde as reagents, but modifications have been proposed in order to reduce its toxicity and increase its efficiency, especially when molecular approaches are involved. It is well known that several factors may interfere in the polymerase chain reaction (PCR) performance. Among them, the amount of DNA present in a sample, since the absence or excess of DNA can interfere in the result, the reagents used in the preparation of the sample, the extraction and dosage of the DNA. In this context, the present work evaluated the influence of the reagents used in the conventional and modified Ritchie method for the identification and quantification of the DNA of cysts of *G. muris*. One gram of stool from male Swiss mice of approximately 60 days old was obtained for the processing of each method variable. The experiment was carried out in triplicate. 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. Finally the group (5) was analyzed by the conventional method (formalin). From the pellet the DNA was extracted in duplicate using the *PureLink PCR Purification*<sup>®</sup> Kit, quantified in *Thermo Scientific Nano Drop 200* and amplified by PCR using primers G18S2 and G18S3. The amplified products were visualized on 4.5% polyacrylamide gel and revealed with silver nitrate. Statistical analysis was performed using the *Software Statistica 8.0*. 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 total amount of DNA was higher for group 3 (33.55 ng /  $\mu$ L), followed by 4 (29, 1 ng /  $\mu$ L), 2 (27.25 ng /  $\mu$ L), 1 (27.25 ng /  $\mu$ L) and 5 (8.4 ng /  $\mu$ L). These results contrast with those obtained by our group in which, in microscopy, the substitution of formaldehyde with water Milli-Q provided higher amount of cysts per gram of faeces, indicating that the amount of cysts may have no relation to the yield of the DNA. DNA was amplified by PCR when obtained from groups 1 to 4, and it was concluded that conventional Ritchie reagents interfere with DNA identification and quantification. Formaldehyde is a PCR inhibitor, also when fecal material is processed suggesting the use of the modified Ritchie method in studies involving molecular biology.