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TECHNICAL REPORT

IDENTIFICATION OF *Cryptosporidium* spp. OOCYSTS IN FECAL SMEARS STAINED WITH HEIDENHAIN'S IRON HEMATOXYLIN

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SUMMARY

There is no paucity of methods for diagnosing *Cryptosporidium* spp. infection. The merits of immunoassays notwithstanding, microscopic identification of *Cryptosporidium* spp. oocysts in fecal samples remains an important diagnostic procedure. It owes the persistence of its use to such characteristics as dispensing with expensive equipment and kits, requiring only basic laboratory facilities, and having a low probability of false positive results when permanent slides are prepared, which can be re-examined in case of doubt. *Cryptosporidium* spp. oocysts can be readily identified in fecal smears prepared according to a regressive iron hematoxylin staining technique. The number of steps and their duration, as well as costs, were reduced to a minimum without loss of image quality and permanence of the preparations.

KEYWORDS: *Cryptosporidium* spp.; Regressive iron hematoxylin staining.

The current availability of reagents and equipment for a wide variety of immunological diagnostic tests for parasitic infections, cryptosporidiosis among them, induces some researchers to relegate microscopic examination to oblivion. Nevertheless, the morphological identification of parasites owes the persistence of its use to such attributes as low probability of false positive results when permanent preparations are made, which can be re-examined in case of doubt or for demonstration purposes. During most of the past century, the identification of protozoan trophozoites and cysts occuring in human feces depended heavily on the quite sharp images produced by Heidenhains's iron hematoxylin staining technique[4]. Traditionally, *Cryptosporidium* spp. oocysts have been identified in stool smears stained by a modified Ziehl-Neelsen technique[1,3]. The rather infrequent use of iron hematoxylin stains at present, in parasitological laboratories, could be attributed to some mostly unnecessary complexities frequently included in the prescriptions for the use of this essentially simple, inexpensive and reliable technique[2]. *Cryptosporidium* spp. oocysts are readily identified in regressive iron hematoxylin-stained fecal smears (Fig. 1). The staining technique recommended for the identification of *Cryptosporidium* spp. oocysts does not require the fixation of wet smears; they can be dried in air before this step, which means a further simplification. Preparations stained with iron hematoxylin, intended for the identification of trophozoites and cysts of various species of protozoa, must be processed while wet, and require the use of a mounting medium before examination under a high-power immersion objective.

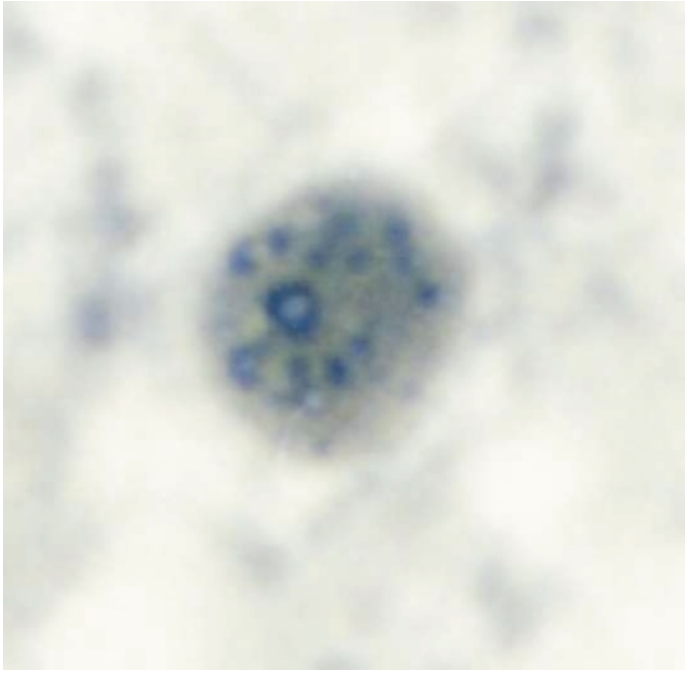

Fig. 1 - *Cryptosporidium* oocyst. Iron hematoxylin stain. Original magnification x7000.

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Fecal smears intended for the search of *Cryptosporidium* spp. oocysts can be processed as follows:

Prepare the smears as usual, allowing them to dry in the air.

Fix the smears by using 10% formalin solution or methyl alcohol; rinse in water (two or three changes, for 1 to 10 minutes); immerse in the mordant solution (1.5 – 2.0% aqueous solution of iron alum); rinse in water, (three or four changes) to remove the excess mordant; stain in 0.25% aqueous hematoxylin solution (about 2 – 10 minutes), depending upon the concentration of "ripe" hematoxylin (hematein); rinse in water (three changes) to remove the excess stain; diferentiate by immersing in the mordant solution (about 1 - 3 seconds); rinse in water (three to five changes), for three to four minutes; after drying, examine under high power immersion objective (90 – 100**×)**.

Recently prepared hematoxylin solutions should be "ripened" (oxidised to hematein, the actual dye) before use. This process is usually achieved by the slow action of atmospheric oxygen but can be accelerated by an oxidising agent, such as sodium iodate. The same result is achieved by hydrogen peroxyde (a few drops). As further oxidising of hematein produces a substance that is not a stain, a too large amount of an oxidising agent or storage for long periods destroys the staining properties of hematoxylin solutions.

A regressive iron hematoxylin staining has been described: the smears are first overstained and then differentiated, i.e. the excess staining substance is removed. This is an important step in this process, as it controls the contrast of the resulting images. The preparations usually remain unaltered for many years. Increasing the duration of the final washing process to remove alum residues from the smears improves the stability of the stain.

RESUMO

Identificação de oocistos de *Cryptosporidium* em esfregaços fecais corados pela hematoxilina férrica de Heidenhain

Não há carência de métodos para o diagnóstico da infecção por *Cryptosporidium* spp. Apesar dos méritos dos imunoensaios, a identificação de oocistos em amostras de fezes permanece um importante recurso diagnóstico que deve a persistência de seu uso a certas características, como dispensar o uso de "kits" e equipamentos dispendiosos, exigindo apenas instrumental básico de laboratório, além de oferecer poucas probabilidades de resultados falsos-positivos, uma vez que as preparações permanentes podem ser examinadas novamente em caso de dúvida. Os oocistos de *Cryptosporidium* spp. podem ser facilmente identificados em esfregaços preparados segundo a técnica regressiva de coloração pela hematoxilina férrica. Procurou-se reduzir ao mínimo o número de etapas da técnica e sua duração, assim como o custo, sem perda da qualidade das imagens e da durabilidade das preparações.

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