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TITLE

CHARACTERIZATION OF OPTIMAL SPREADING OF FECAL SEDIMENTS PRODUCED BY HELMINTEX METHOD TO OPTIMIZE AUTOMATED IMAGE ANALYSIS DETECTION OF S.MANSONI EGGS

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ABSTRACT

Egg microscopy is the most commonly employed tool for diagnosing schistosomiasis. A significant advancement in diagnosing schistosomiasis in low-endemic areas was the developing of the Helmintex method (HTX), which isolates S. mansoni eggs based on their interaction with paramagnetic particles in a magnetic field. However, the HTX method involves multiple steps, culminating in a final sediment that must be analyzed by reading an average of 21 slides. In advancing the improvement of the Helmintex method, this study aimed to evaluate the importance of sample spreading on glass slides for microscopy analysis of the sediment after HTX. Different dilutions were tested using an aliquot from the final stage of HTX, Tween20 at various concentrations, and different volumes of 0.9% NaCl solution to investigate the spreading of the sample on the slide. The sample was spread over an area of approximately 8.5 cm² on a clean glass slide. Pre-selected slides, categorized as having good or poor spreading based on fluorescence microscopy observations, were evaluated for the uniformity of sample distribution using image analysis. Eight random images were captured and analyzed in terms of the area and diameter (measured in micrometers) occupied by granules on the slide for all fragments, using the ImageJ software. It was observed that slides with good spreading covered larger areas than those classified as having poor spreading. An experimental apparatus was created to measure the lux that passes under the glass slide, after analyzing the data from the photometer, we observe that sheets classified as "good" have an average light intensity of 427.5 LUX, while those classified as "bad" have an average of 790.03 LUX. The luximeter measures the amount of light passing through a square meter. This result is analogous to granulometry: good-quality sheets, with their uniform structure, allow less light to pass through, whereas bad-quality sheets, with their irregularities and aggregate disorder, permit more light to pass through.Under the microscope, poorly spread slides exhibited the formation of clusters, resulting in several small areas. Consequently, the spreading of the final HTX sediment was standardized by applying a 30 µL aliquot of the final HTX sediment to the glass slide, followed by the addition of 90 µL of 5% Tween20 solution and 20 µL of 0.9% NaCl solution. It is crucial to note that the quality of the sample spread on the slide can affect the identification of S. mansoni eggs. If the sample is poorly spread and contains aggregates of debris, the eggs may not be visible. Standardizing the sample spreading on the slide is essential for accurate and efficient identification, especially considering the ongoing development of automated image analysis systems. Proper distribution prevents the aggregation of fecal material and plant residues, facilitating specific readings and ensuring precise results.

KEYWORDS

Helmintex; Spreading; Schistosomiasis; Granulometry

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