# Detection of intestinal parasites by use of the cuvette-based automated microscopy analyser sediMAX®

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## Abstract

Microscopy is the reference method for intestinal parasite identification. The cuvette-based automated microscopy analyser, sediMAX 1, provides 15 digital images of each sediment sample. In this study, we have evaluated this fully automated instrument for detection of enteric parasites, helminths and protozoa. A total of 700 consecutively preserved samples consisting of 60 positive samples (50 protozoa, ten helminths) and 640 negative samples were analysed. Operators were blinded to each others' results. Samples were randomized and were tested both by manual microscopy and sediMAX 1 for parasite recognition. The sediMAX 1 analysis was conducted using a dilution of faecal samples, allowing determination of morphology. The data obtained using sediMAX 1 showed a specificity of 100% and a sensitivity of 100%. Some species of helminths, such as *Enterobius vermicularis*, *Strongyloides stercolaris*, the *Ancylostoma duodenale*/*Necator americanus* complex, and schistosomes were not considered in this work, because they are rare in stool specimens, are not easily detectable with microscopy analysis, and require specific recovery techniques. This study demonstrated for the first time that sediMAX 1 can be an aid in enteric parasite identification.



**Keywords:** Digital images, enteric, helminths, identification, protozoa

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## Introduction

Parasitic infections are a major cause of disease and health problems in humans worldwide. It is estimated that around 3.5 billion people harbour parasites and that these cause illness in 450 million [1–9]. Diarrhoea, stomach bloating and digestive disorders are the most common symptoms. Other signs include anaemia, asthma, constipation, fatigue, low immune defences, nervousness and skin rash. There are 3200 varieties of parasites divided into two major groups, the helminths and the protozoa. Helminths, including nematodes (roundworms), cestodes (tapeworms) and trematodes (flatworms), usually cannot reproduce in the human body and rarely cause mortality [1,8]. Protozoa can multiply inside the human body and are widespread in water supplies, infecting a significant proportion of the human population, especially across underdeveloped areas of the world [3,9]. Due to overcrowding and unsanitary conditions, these parasites present a serious health threat and challenging solutions [6,9]. Furthermore, globalization and intense migration have induced the spread of infectious diseases, expanding the range of the human parasites involved [8]. Accurate diagnosis of parasitic infections generally depends on macroscopic or microscopic examination of properly collected and preserved samples [10]. Numerous methods have been described for the recovery and identification of parasites in stool specimens, some of which are useful for detection of a large variety of species, whereas others detect only a particular species [10]. In this work, we applied the sedimentation technique with ethyl acetate concentration that is widely used in most laboratories for both its simplicity and



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suitability in recovering most protozoan cysts, helminth eggs and larvae [11–16].

Manual microscopy is the diagnostic reference method for intestinal parasite detection. However, low parasite density, stool sample number, and the permanent staining necessary for recovery and identification of protozoa, limit microscopic analysis [10,17–19]. Manual microscopy is also labour-intensive, time consuming and requires a high level of technical expertise for optimal interpretation. Furthermore, the laboratory very rarely receives the information required to determine if parasite screening is requested in the clinical context of gastrointestinal complaints or as part of the evaluation of a returning traveller, immigrant, or patient before transplantation. Indeed, diagnosis of parasite infections is hampered by frequent inappropriate test ordering, yielding long turnaround times and limiting the clinical utility of the test. For these reasons, there is a pressing need for newer diagnostic test options to replace the traditional microscopic approaches. Recently developed nucleic acid amplification tests, although expensive, are automated, rapid and show superior accuracy in the search for enteric parasites [19]. Whereas, there is an ever-increasing utilization of automated haematology analysers for blood parasite detection, automated analysers for faecal parasite identification have not yet been developed because of the nature of the sample. A few years ago, a walk-away automated urine sediment microscopy analyser, the sediMAX 1, was introduced for identification and counting of particles in digital images of urine sediment [20]. The sediMAX 1 microscope has an attachment for a digital camera, which takes 15–20 images of each sample, with image magnification approximating to 400× enlargement. All images are analysed by a high-quality image-processing software that is able to detect and classify the particles in urine as blood cells, epithelial cells, crystals, bacteria, yeasts, sperm and mucus, and can be accessed from remote locations [20,21].

The sediMAX 1 has not yet been approved for detection of intestinal parasites, but it could aid conventional microscopy in recognizing and identifying parasitic protozoa and helminths from human specimens. Manual review of digital images would yield a substantial reduction in the number of time-consuming steps necessary for manual microscopy. The purpose of this study was to evaluate the performance of sediMAX 1 in the detection of intestinal parasites in stool samples compared with manual microscopic analysis.

## Materials and Methods

### Collection and preservation of stool samples

Seven hundred faecal specimens for the screening of intestinal parasites (640 negatives and 60 positives) were obtained from June 2013 to June 2014. All patients collected and preserved samples in three different tubes, each containing Universal Fixative solution (TOTAL-FIX®, Medical Chemical Corporation, Torrance, CA, USA), which is free of mercury, formaldehyde and polyvinyl alcohol—necessary to perform adequate ova and parasite evaluation [10–18]. Subsequently, the samples were concentrated using the closed concentration system based on ethyl acetate sedimentation according to the manufacturer (SED-CONNECT™ closed concentration kits; Medical Chemical Corporation). This concentration method is recommended because it is the easiest to perform; it allows recovery of the broadest range of organisms from all protozoa to helminth eggs and larvae present, and is least subject to technical error.

### sediMAX® instrument: materials and technical details

Sequential samples for routine intestinal parasite screening were randomized and analysed both by microscopy, and by sediMAX 1 (77 Elektronika, Budapest, Hungary, distributed by Menarini Diagnostics, Italy). Operators were blinded to each other's results. Initially, a routine scan for parasites was performed with examination at low power (25×), and with higher magnification (40×) when necessary. The analogous examination was performed on sediMAX 1 using an image magnification of about 400×. Before sediMAX 1 analysis, faecal specimens were diluted (1 : 20) with 0.45% saline solution due to the solid or semi-solid nature of the stool sample. The sediMAX 1 requires 2 mL of sample in a test tube. Two hundred microlitres of this sample are pipetted into a cuvette and centrifuged at 1200 g for 10 s to force the particles into one plane at the bottom of the cuvette. The internal digital camera then takes 15 images, representing the examination of a total 2.4 µl. Each sample was analysed once to determine parasite concentration; it was then further analysed from two to four times, taking from 15 to 60 images. The samples were then evaluated, and the images acquired by sediMAX 1 were reviewed on screen for the presence of parasites by one technician and two clinicians, each blinded to the others' results.

### Statistical analysis

Sensitivity, specificity and statistical analysis were performed with MedCalc Statistical Software version 14.10.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014) [22]; $p < 0.05$ was considered to be significant.

## Results and Discussion

This is the first study to use sediMAX 1 for intestinal parasite detection in routine stool samples. The sediMAX 1 was