

A comparative study of the TF-Test[®], Kato-Katz, Hoffman-Pons-Janer, Willis and Baermann-Moraes coprologic methods for the detection of human parasitosis

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*This study compares the diagnostic accuracy of the TF-Test[®] (TFT) for human parasitosis with results obtained using the traditional Kato-Katz (KK), Hoffman-Pons-Janer (HPJ), Willis and Baermann-Moraes (BM) techniques. Overall, four stool samples were taken from each individual; three alternate-day TFT stool samples and another sample that was collected in a universal container. Stool samples were taken from 331 inhabitants of the community of Quilombola Santa Cruz. The gold standard (GS) for protozoa detection was defined as the combined results for TFT, HPJ and Willis coproscopic techniques; for helminth detection, GS was defined as the combined results for all five coproscopic techniques (TFT, KK, HPJ, Willis and BM). The positivity rate of each method was compared using the McNemar test. While the TFT exhibited similar positivity rates to the GS for *Entamoeba histolytica/dispar* (82.4%) and *Giardia duodenalis* (90%), HPJ and Willis techniques exhibited significantly lower positivity rates for these protozoa. All tests exhibited significantly lower positivity rates compared with GS for the diagnosis of helminths. The KK technique had the highest positivity rate for diagnosing *Schistosoma mansoni* (74.6%), while the TFT had the highest positivity rates for *Ascaris lumbricoides* (58.1%) and hookworm (75%); HPJ technique had the highest positivity rate for *Strongyloides stercoralis* (50%). Although a combination of tests is the most accurate method for the diagnosis of enteral parasites, the TFT reliably estimates the prevalence of protozoa and selected helminths, such as *A. lumbricoides* and hookworm. Further studies are needed to evaluate the detection accuracy of the TFT in samples with varying numbers of parasites.*

Key words: coprologic diagnostic methods - helminth infection - protozoan infection - TF-Test

Parasitic diseases are classified as neglected tropical diseases by the World Health Organization and affect one billion people worldwide (WHO 2006). The prevalence of parasitosis is correlated with deficient basic sanitation, poor hygienic habits and low-quality health care assistance. In spite of effective sanitary measures and the use of broad-spectrum antiparasitic treatments for the reduction of the prevalence of geo-helminthiasis, parasites that infect humans still represent an important sanitary problem in many regions of the world (Keiser & Utzinger 2008). Enteric protozoan infections add complexity to public health management because the high toxicity of antiprotozoal agents limits the implementation of mass treatment strategies (Ferreira & Marçal Junior 1997).

The majority of detection methods routinely used for the diagnosis of intestinal helminths and protozoan infections in humans are inexpensive and simple to perform (De Carli 2001). However, these tests have limitations, particularly regarding to their sensitivity. Thus,

the use of more than one method is necessary to detect different parasitic forms, especially under conditions of low parasite burdens (Sudré et al. 2006).

To provide a better coprologic method for clinical diagnostic and research purposes, a new centrifugal concentration technique, known as the TF-Test[®] (TFT) (Immuno-Assay[®], Itupeva, Brazil), is now commercially available. The TFT is useful for the diagnosis of human and animal enteric parasitosis because of its greater sensitivity compared with other techniques (Lumina et al. 2006). This method is extremely efficient because it incorporates double filtration, centrifugation and sedimentation at a low cost and with easy handling. The TFT is recommended in situations in which the stool samples originate from areas that are geographically distant from the clinical or research laboratories where analyses occur. Samples are collected in a preservative medium, providing a leeway of up to 30 days between collection and analysis. In this context, this study aims to compare the diagnostic accuracy of the innovative TFT for human parasitosis with the conventional Kato-Katz (KK), Hoffman-Pons-Janer (HPJ), Willis and Baermann-Moraes (BM) techniques.

SUBJECTS, MATERIALS AND METHODS

Subjects and area of study - The population studied included all 377 inhabitants of the Quilombola Santa Cruz community of Brazil. Quilombola Santa Cruz is situated in a rural area of the Ouro Verde de Minas mu-

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nicipality in the northeast region of the state of Minas Gerais (MG); this community is 480 km from Belo Horizonte, the capital city of MG.

This population was selected and invited to participate in this comparative study because it is situated in a region where gastrointestinal parasitosis and schistosomiasis in particular, is highly prevalent. For this reason, this area is included in the Schistosomiasis Control Programme of the National Health Ministry. According to official health system data, the local mortality rate attributed to parasitic and infectious diseases was 5.1% in 2008 (DATASUS 2011).

Stool sample collection - Three faecal samples from each subject were collected on alternate days in plastic tubes containing formaldehyde as the preservative medium for the TFT assay. For the KK, HPJ, Willis and BM assays, one additional faecal sample was subsequently collected in a sterile universal container without a preservative solution and was stored at 4°C according to the protocol described by Oliveira et al. (2002). TFT assays were performed within seven days of sample collection. The assays that utilised unpreserved faecal samples were processed within 24 h of collection.

Subjects were divided into groups of 80 people and stool samples from each group were collected and analysed each week. Sample processing and analysis took place in the following order: HPJ (n = 270), BM (n = 271), Willis (n = 292), KK (n = 270) and TFT (n = 308). All of the testing procedures were completed within eight weeks.

Parasitological techniques - For each stool sample, two slides were examined for the detection of parasites by two investigators using optical microscopy (Nikon, Japan) at 100X and 400X magnifications; samples were examined after being concentrated using the conventional coprologic techniques and the commercial TFT. The results of all tests were compared to the gold standard (GS) parameter for each sample. Samples that tested positive using at least one coprologic technique were considered to be positive according to the GS.

Conventional coprologic techniques - Stool sample concentrations were performed using five conventional techniques: KK, HPJ, Willis and BM methods. The KK (Katz et al. 1972) technique is a semi-concentration technique used to qualitatively detect parasite eggs. The commercial Helm-Test Kit (Labmaster Ltd, Belo Horizonte, MG, Brazil) was used according to manufacturer instructions. Briefly, stool samples were compressed using mesh to concentrate the samples and to separate the detritus. Next, samples were transferred to a slide through a perforated plate that defines a uniform amount of faeces. The perforated plate was subsequently removed and the stool samples were covered by a cellophane slide moistened in glycerin. The HPJ (Hoffman et al. 1934) technique utilises spontaneous faecal sedimentation. In this method, faecal samples are diluted in water and filtered through a gauze strip into a conical sedimentation glass. The HPJ method was used to detect the presence of helminth eggs and larvae and protozoa cysts. This technique is widely used in epidemiological studies, due to its low cost. The Willis (Willis 1921) tech-

nique is a flotation method based on the ability of helminth eggs to float on the surface of a saturated sodium chloride solution with a density of 1.20 g/mL and to adhere to glass. In this technique, a saturated solution with the emulsified faeces is deposited in a round-bottomed flask and a meniscus is formed on the surface. Next, the flask is covered with a slide. After several minutes, the slide is removed and examined under a microscope. The BM technique (Moraes 1948) is used for the detection of nematode larvae in faecal samples and is based on the thermotropism and hydrotropism of the larvae, which exhibit a tendency toward sedimentation. This technique consists of placing the faeces in contact with warm water at 40-45°C for 1 h such that the larvae present in the faeces tend to migrate into the warmer, liquid media and settle at the bottom of the flask. Differentiation between *Strongyloides stercoralis* and hookworm larvae was achieved by analysing the morphological characteristics of the buccal vestibules and by the presence or absence of the developing genital primordium larvae.

TF-Test - All laboratory samples processed using the TFT kit were handled according to manufacturer instructions. A TFT kit includes three collection tubes containing neutral buffered formalin (patient kit) and a conical flask with a double-filtering system with 400 µm and 200 µm meshes for centrifugation (laboratory kit) for parasite concentration. The TFT kit was developed to process simultaneously three faecal samples collected on alternate days. Approximately 1 g of faeces from each patient was added to each of the three collection tubes, which were vigorously agitated for homogenisation. Each tube received a drop of neutral detergent plus 3 mL ethyl acetate and was attached to a conical flask for centrifugation for 1 min at 1,500 rpm. The supernatants were discarded and the sediments were homogenised with normal saline solution. For each sample, one drop of the homogenised material was transferred to a microscope slide containing one drop of Lugol's solution. Slides were subsequently observed using an optical microscope.

Statistical analysis - For data analysis, the combined results for the three (n = 261) and five (n = 213) coproscopic techniques employed were assumed as the GSs for the detection of protozoa and helminths, respectively. The positivity rates of the three techniques (TFT, HPJ and Willis) for the detection of enteric protozoan infections and the five techniques (TFT, HPJ, Willis, KK and BM) for detection of intestinal helminthiasis were calculated. Those samples with an insufficient amount of stool for the execution of all tests were excluded from the analysis. The proportions of positive results were compared using the paired McNemar χ^2 test; the GS was utilised as the reference. The prevalence of parasitic infections was calculated from the positive results of at least one of the techniques.

Ethics - This study adheres to all of the current ethical recommendations for research in humans. The population received specific parasitic treatment and medical attention after the identification of the infections and the results were reported to the municipal authorities.

RESULTS

From the total population of 377 subjects, 331 provided stool samples for examination (Fig. 1). Please consider a positive result in any of the methods employed as a positive reference for parasitic infection ("All tests"). For statistical analysis, samples that tested positive using any of the testing methods employed were considered positive in the "All tests" category. Positive rates for the detection of enteric protozoa and helminths for the individual tests were then compared with the "All tests" data. These results are presented in Fig. 2. The TFT had the highest positivity rate for diagnosing protozoa (38.3%), while the KK technique had the highest positivity rate for helminth detection (34.1%). However, the prevalence for the TFT and KK techniques were lower than the 43.5% for protozoan and 45.6% for helminth infections showed in the "All tests" category.

The prevalence for each parasite species and the positivity rates for the methods employed in relation to the GS are depicted in Table. For protozoa detection, the TFT exhibited similar positivity rates to the GS for *Entamoeba histolytica/dispar* (82.4%) and *Giardia duodenalis* (90%). In contrast, the HPJ and Willis techniques exhibited significantly ($p < 0.05$) lower positivity rates compared with the GS. For helminth detection, all tests exhibited significantly ($p < 0.05$) lower positivity rates

compared with the GS. The KK method had the highest positivity rate for diagnosing *Schistosoma mansoni* (74.6%), while the TFT had the highest positivity rates for diagnosing *Ascaris lumbricoides* (58.1%) and hookworm (75%); the HPJ technique had the highest positivity rate for diagnosing *S. stercoralis* (50%).

DISCUSSION

Several coprologic techniques have been utilised in parasitic disease diagnostic tests; however, none was capable of detecting all parasitic infections when employed separately. In this scenario, it would be advantageous to combine techniques with the capacity to detect a broad range of intestinal parasites in a standard procedure. The TFT was recently developed for the di-

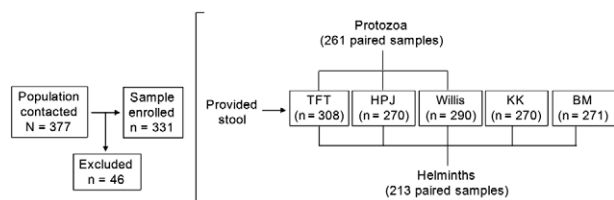


Fig. 1: trial diagram of number of persons contacted and enrolled, stool samples provided and examined by TF-Test® (TFT), Kato-Katz (KK), Hoffman-Pons-Janer (HPJ), Willis, Baermann-Moraes (BM) methods.

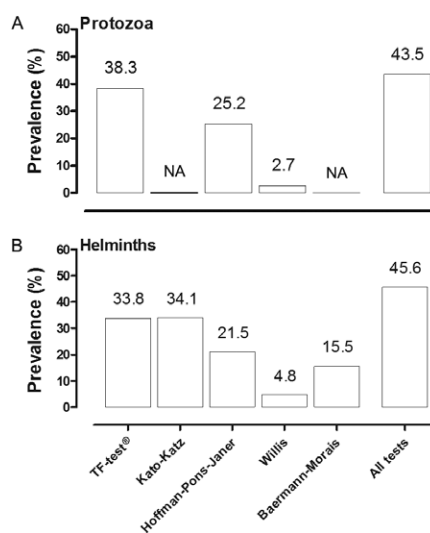


Fig. 2: prevalence of protozoan (A) and helminthic infections (B) determined by different diagnostic methods. The prevalence of parasitic infections was obtained by a positive result in at least one technique (All tests). NA: not applicable.

TABLE

Comparison of the positivity rate according to parasitological method in stool samples (n = 331) from Quilombola Santa Cruz, Ouro Verde de Minas, Minas Gerais, Brazil, 2007

| Parasitological test | GS ^a N (n) | TFT n (%) | KK n (%) | HPJ n (%) | Willis n (%) | BM n (%) | All tests N (n) prevalence |
|-------------------------------------|--------------------------|------------------------|-------------|--------------|-----------------|-------------|-------------------------------|
| <i>Entamoeba histolytica/dispar</i> | 261 (17) | 15 (82.4) ^b | NA (NA) | 5 (29.4) | 1 (5.9) | NA (NA) | 315 (20) 6.35 |
| <i>Entamoeba coli</i> | 261 (60) | 48 (80) | NA (NA) | 39 (65) | 4 (6.7) | NA (NA) | 315 (69) 21.9 |
| <i>Endolimax nana</i> | 261 (60) | 47 (78.3) | NA (NA) | 24 (40) | 3 (5) | NA (NA) | 315 (74) 23.5 |
| <i>Giardia duodenalis</i> | 261 (30) | 27 (90) ^b | NA (NA) | 10 (33.3) | 2 (6.7) | NA (NA) | 315 (33) 10.5 |
| <i>Schistosoma mansoni</i> | 213 (73) | 45 (61.6) | 54 (74.6) | 25 (34.2) | 0 (-) | 7 (9.6) | 331 (108) 32.6 |
| <i>Ascaris lumbricoides</i> | 213 (31) | 18 (58.1) | 13 (41.9) | 17 (54.8) | 6 (19.4) | 14 (45.2) | 331 (38) 11.5 |
| Hookworm | 213 (24) | 18 (75) | 1 (4.2) | 10 (41.7) | 7 (29.2) | 4 (16.7) | 331 (29) 8.8 |
| <i>Strongyloides stercoralis</i> | 213 (24) | 9 (37.5) | 0 (-) | 12 (50) | 1 (4.2) | 10 (41.7) | 331 (30) 9.1 |

a: defined by paired results obtained for all tests; b: McNemar test not significant in relation to Gold Standard (GS); BM: Baermann-Moraes; HPJ: Hoffman-Pons-Janer; KK: Kato-Katz; NA: not applicable; TFT: TF-Test®.

agnosis of intestinal parasites and exhibits remarkable results compared to conventional coprologic techniques (Gomes et al. 2004). This method favourably combines different technical procedures, including centrifugation, filtration and sedimentation.

In this study, we found that the TFT had higher positivity rates in detecting both protozoa and helminths in human stool samples compared with the conventional KK, HPJ, Willis and BM coprologic techniques. Additionally, Gomes et al. (2004) showed that the TFT had a higher diagnostic positivity rate compared to the Coprotest Kit for human coprologic diagnosis. These findings were not confirmed in dogs (Katagiri & Oliveira-Sequeira 2010). However, in the dog study, only a single faeces sample was collected and analysed. In this study and in a study from Gomes et al. (2004) examining human parasitosis, three faecal samples were collected on alternate days. More recently, Lumina et al. (2006) adapted the TFT for the quantitative diagnosis of goat enteric parasites and compared the adapted test with the Gordon-Whitlock method described by Ueno and Gonçalves (1998). The authors obtained good concordance between the techniques. Because the TFT has the advantage of employing preservatives, it can be employed in areas that are geographically distant from the testing laboratories.

In this study, we found that the evaluated coprologic techniques for the detection of human intestinal parasites had variable efficacies in relation to the TFT. The spontaneous sedimentation assay (HPJ) is widely employed in clinical laboratories, due to its capacity to detect both protozoa and helminths. However, with the exception of *S. stercoralis* larvae, the HPJ test is less effective in detecting both helminths and protozoa compared to the TFT. Although both techniques involve sedimentation as the concentration method, the higher positivity rates of the TFT could be explained by the additional use of centrifugation. Additionally, the TFT employs ethyl acetate, a detergent solution and two filter nets that combine to result in a cleaner preparation that facilitates the identification of parasitic forms (Katagiri & Oliveira-Sequeira 2010).

The BM method is able to detect nematode larvae even when parasite burdens are low (Oliveira et al. 2002). In this study, the BM had a lower positivity rate compared with the HPJ method for the detection of parasite larvae. In addition, the extensive use of BM is not recommended because of its low positivity rates for other parasites. However, when combined with the modified Faust method, the BM technique can be advantageously employed in epidemiological inquiries and in routine laboratory stool examinations for the detection of enteric helminths (Chaves et al. 1979).

Another coprologic method evaluated was the Willis technique. This assay is the most commonly used technique for detecting light structures, such as protozoan cysts and hookworm eggs (Cerqueira et al. 2007). Surprisingly, the TFT detected 2.5 times more positive stool samples for hookworm than the Willis technique, which is the reference test for hookworm detection. Additionally, the spontaneous sedimentation assay (HPJ) also had a higher positivity rate for hookworm compared with the Willis method. These findings have been confirmed by

other authors who reported higher detection sensitivity for hookworm using the HPJ method compared with the Willis technique (Cerqueira et al. 2007).

However, the TFT technique had a lower positivity rate than the KK method for the detection of *S. mansoni* eggs. In contrast, the TFT positivity rates for the detection of *A. lumbricoides* and hookworm infections were higher compared with the KK technique. According to Brown et al. (2003), hookworm eggs clear rapidly in the KK procedure and are not usually visible unless the smears are examined immediately after preparation.

These results are interesting, especially considering that the capacity of coprologic techniques to detect a broad range of intestinal parasites is important when caring for individual patients, especially in regions where protozoa, hookworm and *S. stercoralis* are prevalent. In summary, the TFT for human coprologic diagnosis had higher positivity rates for the detection of both protozoan and helminths infections compared with several of the conventional concentration techniques evaluated. However, further evaluation of the TFT is necessary, especially because no methods specific for protozoa detection were employed in this study. Because the presence of live and active larvae enhances the sensitivity of the BM technique, the efficacy of this assay method is decreased when samples are refrigerated prior to processing. Additionally, test performances vary under different conditions and no methods were employed for the quantification of parasite burdens. The use of quantitative methods would permit the stratification of results and the evaluation of the performances of each method depending on parasite abundance. Future studies employing quantitative methods, especially those planned for stratification analysis, will be necessary to establish the performance differences of the methods more clearly at varying levels of parasite burdens.

Based on the findings in this study, we conclude that the TFT furnishes a reliable estimate of the prevalence of protozoan infection in endemic areas. At the same time, the TFT exhibits higher or comparable positivity rates compared to other techniques utilised for helminth detection. Therefore, the TFT method is of fundamental importance for the evaluation of sanitation interventions for the control of enteral parasitosis.

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