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Evaluation of a Novel Kit (TF-Test) for the Diagnosis of Intestinal Parasitic Infections

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Intestinal parasitic infections are currently a source of concern for Public Health agencies in developing and developed countries. Since three ovum-and-parasite stool examinations have been demonstrated to provide sensitive results, we designed a practical and economical kit (TF-Test) that is now commercially available (Immunoassay Com. Ind. Ltda., São Paulo, Brazil). This kit allows the separate collection of three fecal specimens into a preservative solution. The specimens are then pooled, double-filtered, and concentrated by a single rapid centrifugation process. The TF-Test was evaluated in four different laboratories in a study using 1,102 outpatients and individuals living in an endemic area for enteroparasitosis. The overall sensitivity found using the TF-Test (86.2–97.8%) was significantly higher ($P<0.01$) than the sensitivity of conventional techniques such as the Coprotest (NL Comércio Exterior Ltda, São Paulo, Brazil) and the combination of Lutz/Hoffman, Faust, and Rugai techniques (De Carli, *Diagnóstico Laboratorial das Parasitoses Humanas. Métodos e Técnicas*, 1994), which ranged from 48.3% to 75.9%. When the above combined three specimen technique was repeated with three specimens collected on different days, its sensitivity became similar ($P>0.01$) to that of the TF-Test. The *kappa* index values of agreement for the TF-Test were consistent ($P<0.01$), being higher and ranking in a better position than conventional techniques. The high sensitivity, cost/benefit ratio, and practical aspects demonstrate that the TF-Test is suitable for individual diagnosis, epidemiological inquiries, or evaluation of chemotherapy in treated communities. J. Clin. Lab. Anal. 18:132–138, 2004.

Key words: TF-Test; parasite-enrichment process; pooled three fecal specimen examinations; intestinal parasitic infections

INTRODUCTION

The prevalence of intestinal parasitic infections is high, mostly in tropical and subtropical developing countries. The World Health Organization (1) estimates that about 3.5 billion people in the world have single or multiple intestinal parasitoses. Also, intestinal parasitoses have become a public health concern in developed countries because of the increase in intercontinental travel and immigration and the increase in the number of immunocompromised subjects. Physicians in developed and developing countries are now requesting frequent stool examinations for intestinal parasites, or they are recommending at least one stool examination per year, especially for immunocompromised patients (2).

The conventional techniques involving ovum-and-parasite (O&P) examination have been proven to miss

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many pathogenic parasites (3). Thus, in order to confirm the presence of intestinal parasites, it has been shown that three stool specimens are needed in routine laboratory examinations (4). In addition, in a high-prevalence setting, at least two examinations have been considered to be necessary (5,6).

In an attempt to improve the efficiency of stool examination techniques, several concentration procedures have been suggested, either by pooling three formalin-preserved stool specimens for conventional techniques (7) or by using some commercially available concentration device suitable for concentrating one stool specimen (8).

We have recently designed a practical and economical kit (TF-Test) that is now manufactured by Immunoassay Com. Ind. Ltda. (São Paulo, Brazil). This kit allows the collection of stool specimens separately into a preservative solution on three different days. The specimens are then pooled by a 1-min centrifugation process, double-filtered, and concentrated before parasite identification by standard light microscopy.

In this study, evaluation of the TF-Test's diagnostic features is presented in comparison with conventional techniques for an O&P examination, performed at four reference laboratories belonging to universities located in different cities in the State of São Paulo, Brazil.

MATERIALS AND METHODS

Stool Specimens

A total of 1,102 subjects were studied and three stool specimens were collected from each individual for the TF-Test. In addition, one more specimen was collected for the conventional technique used in each of three laboratories in the State of São Paulo. In the first laboratory (A) located in the City of Taubaté (Laboratory of Parasitology, Department of Biology, University of Taubaté), specimens were collected from inhabitants of the rural zone, an endemic area for enteroparasitosis. In the second laboratory (B), in the City of Botucatu (Laboratory of Clinical Analyses, Clinical Hospital, Paulista State University), specimens were collected from outpatients. In the third laboratory (C), in the City of Campinas (Laboratory of Clinical Parasitology, School of Medical Sciences, State University of Campinas), specimens were also collected from outpatients. In the fourth laboratory (D) located in the City of São Paulo (Laboratory of Parasitology, Clinical Hospital, Medical School, University of São Paulo), three stool specimens were collected for the TF-Test and three specimens for each of the three standard O&P techniques.

This project was submitted to the Ethics Committee of each university, and written informed consent was obtained from the subjects who agreed to participate in the project.

TF-Test

The parasite enrichment device (Fig. 1) was made of disposable and recyclable plastic (polypropylene) and consisted of three vials for specimen collection, each containing 5 mL preservative solution that displayed a fill-to line in order to permit the patient to visually check whether or not the amount of collected stool specimen was adequate. Several fixatives were available, but all participating laboratories used the traditional formalin-buffered solution. About 1.0 g stool specimen was collected from each vial with a scoop connected to the cap, and three specimens were obtained on alternate days, or within a week. After collecting each specimen, the patient was asked to homogenize the vial by moderate shaking in order to ensure parasite fixation and the maintenance of their morphological structure. In the laboratory, 2 mL ethyl-acetate and a drop of detergent were added to each vial. After homogenization, all the vials were coupled to a double-filtration system attached to a conical centrifuge tube and submitted to a 1-min centrifugation ($500 \times g$). The centrifuge tube was then detached from the system, the supernatant was discarded, 10 drops of saline were added to the sediment, and one drop of the sediment suspension was placed on a slide. However, depending on sediment concentration, one additional drop of saline was added before examination for the purpose of detecting parasites by routine microscopy.

Conventional Techniques for Stool Examination

The conventional techniques for O&P examination differed according to the laboratory: laboratories B and C used only the Coprotest, previously known in Brazil as Total test (NL Comércio Exterior Ltda.) (9), which consists of one vial for collecting one stool specimen; laboratory A used a combination of the Coprotest and Kato-Katz (10); and laboratory D used a combination of the Lutz/Hoffman, Faust, and Rugai techniques (11).

Statistical Analysis

The results of the TF-Test were evaluated in comparison with those of the routinely used techniques. The positivity found by the combination of all techniques used in each laboratory was considered to be the reference value. The positivity found by the TF-Test was compared with the standard techniques by the z-test of proportions (12). Also, 95% confidence intervals were calculated for sensitivity or specificity (13). The efficiency of the techniques was also

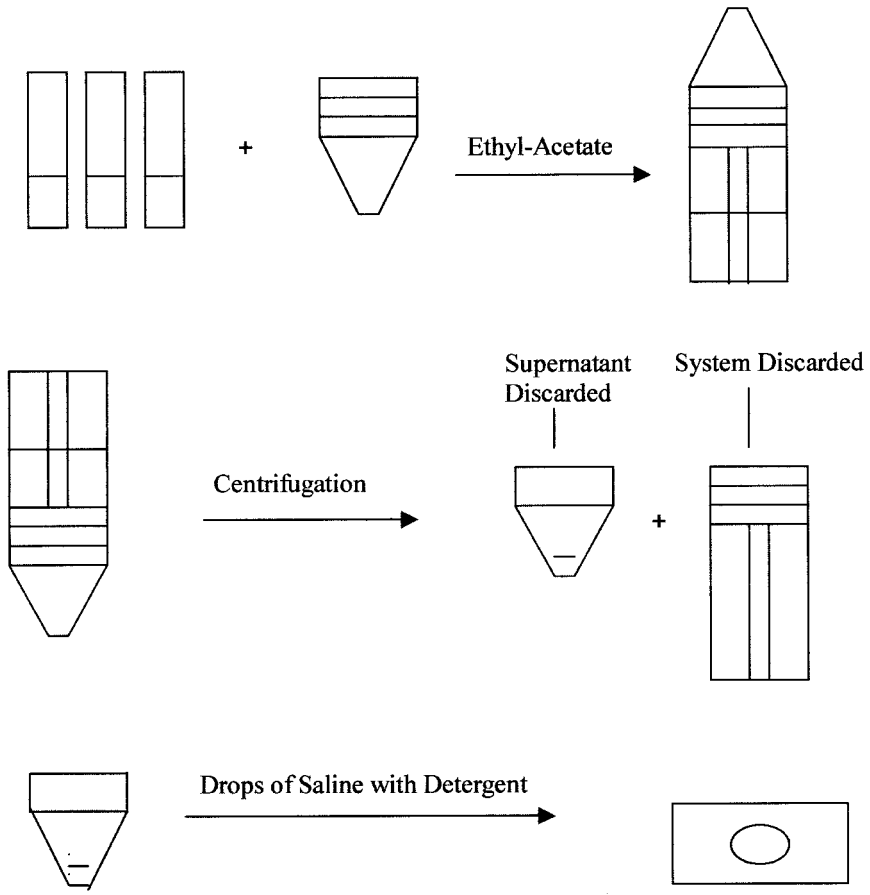

Fig. 1. Schematic procedure for the TF-Test: three plastic tubes with stool specimens are coupled to an assembled system of double-filter and centrifuge tube. The whole system is inverted and centrifuged. The centrifuge tube is detached, the supernatant is discarded, and some drops of saline with detergent are added to the sediment. The concentrated mixture is placed on a microscope slide with a coverslip. Parasites are identified by standard light microscopy.

determined in terms of the *kappa* (κ) index of agreement (14) by testing the consistency of κ (15) and its rank, based on the strength of the κ index (16), and defined as follows: *poor* for values ranging from 0 to 2.0, *slight* for values from 0.21 to 0.40, *moderate* for values from 0.41 to 0.60, *substantial* for values from 0.61 to 0.80, and *almost perfect* for values from 0.8 to 1.0.

RESULTS

TF-Test and Conventional Techniques

The results regarding the number of infected subjects, type of infection (single or multiple), and the number of different parasite species detected by the TF-Test and by the conventional techniques are presented in Table 1. Each laboratory had its own reference data corresponding to the overall positive and negative results obtained by the combination of the conventionally used technique(s) including the TF-Test.

In laboratories A, B, C, and D, intestinal parasitic infections were found at frequencies ranging from 14.3–60.5%. The TF-Test detected a total of 406 (36.8%) subjects with single or multiple enteroparasitosis, a significantly higher number (obtained $z = 3.25$; critical $z = 2.57$; $P < 0.01$) than the 334 (30.3%) infected subjects detected by conventional techniques. Completely negative results were observed in 660 (59.8%) subjects by all the techniques used with 31.4% (207) of these being from laboratory A, 38.9% (256) from laboratory B, 14.2% (94) from laboratory C, and 15.5% (102) from laboratory D.

Parasite Species

In 443 subjects with single, double, triple, or multiple (≥ 4) infections, a total of 807 intestinal parasitic infections were detected. The positivity found by the TF-Test was 88.1% (711), which was significantly higher (obtained $z = 11.5$; $P < 0.01$) than the positivity of 63.7%

TABLE 1. Results obtained in the study of 1,102 subjects by the TF-Test and by conventional techniques in four different laboratories in the State of São Paulo, Brazil

Laboratory (no. of studied subjects)	Technique	No. of subjects with single or multiple infections (%)	Type of Infection				Total no. of parasite Infections detected[b]
			Single no.	Double no.	Triple no.	Multiple[a] no.	
A	TF-Test	100 (32.4)	76	16	7	1	134
(309)	Coprotest	80 (25.9)	62	14	3	1	104
	Positives	102 (33.0)	77	17	7	1	137
B	TF-Test	35 (11.7)	32	3	0	0	38
(300)	Coprotest	31 (10.3)	29	2	0	0	33
	Positives	43 (14.3)	40	3	0	0	46
C	TF-Test	137 (57.6)	79	26	21	11	242
(238)	Coprotest	112 (47.1)	59	25	14	14	208
	Positives	144 (60.5)	76	30	19	19	275
D	TF-Test	134 (52.3)	42	42	31	18	297
(256)	Three tec.[c]	111 (43.4)	70	32	3	6	169
	Positives	154 (60.2)	49	50	30	25	349

[a]Multiple means ≥ 4 parasitic infections.
[b]In affected subjects.
[c]Combination of the Lutz/Hoffman, Faust, Rugai techniques.

(514) found by conventional techniques (Table 2). In laboratory A, the data provided by the Kato-Katz (10) technique were not included because the positive results were very few and agreed with the Coprotest. In Table 2, the overall protozoan and helminth species identified by the TF-Test and conventional techniques are also presented.

Protozoan infections were observed in 56.7% (625/1102) of subjects, with their frequency being considerably higher than that of helminth infections (16.5%, 182/1102) in four laboratories. Statistical analysis confirmed that the frequency of protozoan infections was significantly higher than that of helminth infections (obtained $z = 12.0$; $P < 0.01$).

Sensitivity and Specificity of the Techniques

The sensitivity of the TF-Test was calculated on the basis of the ability of a technique to detect infections caused by different parasite species in comparison with the reference data. Table 3 shows that the TF-Test presents significantly higher sensitivity (obtained $z =$ and > 3.8; $P < 0.01$) than the conventionally used techniques, but in laboratory B, the TF-Test and Coprotest presented similar sensitivity (obtained $z = 1.25$; $P > 0.01$) due to the low number of positive results. A total of 300 outpatients were studied in laboratory B and only 43 of them were infected with 46 parasite species. Also, in terms of the confidence intervals (95%), the sensitivity of the TF-Test (69.0–91.0%) overlapped the sensitivity of the Coprotest (57.0–83.1%), indicating no difference between these two techniques.

TABLE 2. Parasite species identified by the TF-Test and conventional techniques in the study of 1,102 subjects in four different laboratories in the State of São Paulo, Brazil

Parasite species	TF-test (positive no.)	Routine technique[a] (positive no.)	Total positivity (no.)
Protozoan			
E. nana	13.5% (149)	9.6% (106)	15.7% (173)
E. coli	14.0% (154)	9.5% (104)	15.1% (166)
B. hominis	10.1% (111)	6.8% (75)	11.7% (129)
G. lamblia	8.2% (90)	5.5% (61)	8.4% (93)
I. butschlii	2.5% (28)	1.5% (17)	3.0% (33)
E. histolytica/dispar	1.6% (18)	0.8% (9)	1.9% (21)
E. hartmanni	0.3% (3)	0.5% (5)	0.7% (8)
C. mesnili	0.1% (1)	0.2% (2)	0.2% (2)
Helminth			
Ancylostomatidae	5.3% (58)	4.7% (52)	5.7% (63)
T. trichiura	4.1% (45)	3.2% (35)	4.4% (48)
S. stercoralis	2.8% (31)	2.0% (23)	3.6% (40)
S. mansoni	1.1% (12)	1.1% (13)	1.6% (18)
E. vermiculares	0.8% (9)	0.9% (10)	1.0% (11)
H. nana	0.2% (2)	0.2% (2)	0.2% (2)
Total	64.5% (711)	46.6% (514)	73.3% (807)

[a]Coprotest or a combination of the Lutz/Hoffman, Faust, and Rugai techniques.

Maximum specificity (100%) was found for all the techniques in all four laboratories. In laboratory D, the sensitivity of the Lutz/Hoffman, Faust, and Rugai techniques, each alone or in combination (Table 4), was found to increase significantly (obtained $z \geq 2.7$; $P < 0.01$) after repeating two and three stool collections and O&P examinations. Also, the sensitivity of the

TABLE 3. Sensitivity and specificity of the TF-Test and conventional techniques in the study of 1,102 subjects with different types of intestinal parasitoses in four laboratories in the State of São Paulo, Brazil

Laboratory	Technique	Sensitivity % (positives/total positives)	Specificity % (negatives/total negatives)
A	TF-Test	97.8[a] (134/137)	100 (207/207)
	Coprotest	75.9 (104/137)	100 (207/207)
B	TF-Test	82.6 (38/46)	100 (257/257)
	Coprotest	71.7 (33/46)	100 (257/257)
C	TF-Test	88.0[a] (242/275)	100 (94/94)
	Coprotest	75.6 (208/275)	100 (94/94)
D	TF-Test	85.1[a] (297/349)	100 (102/102)
	Lutz/Hoffman, Faust, and Rugai	48.1 (168/349)	100 (102/102)
A, B, and C	TF-Test	90.4[a] (414/458)	100 (558/558)
	Coprotest	75.3 (345/458)	100 (558/558)

[a] $P < 0.01$.

TABLE 4. Increased sensitivity of the Lutz/Hoffman, Faust, and Rugai techniques and of their combination by repeating the ovum-and-parasite stool examination for a total of 256 patients with intestinal parasitic infections

Technique	Sensitivity (repeat no.)		
	One	Two	Three
Lutz/Hoffman	38.4% (134)[a]	65.6% (229)	86.5% (302)
Faust	25.5% (89)	41.8% (146)	60.7% (212)
Rugai	12.0% (42)	19.5% (68)	30.4% (106)
Lutz/Hoffman, Faust, and Rugai	48.1% (168)	78.8% (275)	89.4% (312)

[a]Positive number.

TABLE 5. Kappa (κ) indices of agreement for different parasitologic techniques ranked according to their strength in four laboratories in the State of São Paulo, Brazil

Laboratory	Technique	κ index[a]	κ rank
A	TF-Test	0.976	*Almost perfect*
	Coprotest	0.785	*Substantial*
B	TF-Test	0.877	*Almost perfect*
	Coprotest	0.799	*Substantial*
C	TF-Test	0.782	*Substantial*
	Coprotest	0.601	*Moderate*
D	TF-Test	0.715	*Substantial*
	Lutz/Hoffman, Faust, and Rugai	0.293	*Slight*
A, B, and C	TF-Test	0.906	*Almost perfect*
	Coprotest	0.765	*Substantial*

[a]All κ values were consistent ($P < 0.01$).

combination of these techniques improved gradually and significantly achieving 89.4%, which did not differ significantly (obtained $z = 1.58$; $P > 0.01$) from the TF-Test (85.1%) in the same laboratory.

Kappa (κ) Index of Agreement

The κ index indicates the agreement of positive and negative results between a technique under evaluation and the reference data, considered here as true diagnoses. In all laboratories, the TF-Test ranked in a better position than the conventional techniques (Table 5). All the κ indices obtained were consistent because the obtained z-values were all higher than 3.89 ($P < 0.01$).

DISCUSSION

The techniques currently used for O&P examination are usually highly specific, but tend to yield false-negative results. Thus, the improvement of these techniques for the identification of parasites in stool specimens is imperative in order to obtain sensitive results. Most Public Health laboratories from developing countries are interested in stool examination techniques because they focus on the unequivocal diagnosis of intestinal parasitosis at low cost. Thus, in an attempt to satisfy the expected diagnostic features, the TF-Test was designed to deal with this matter.

Parasitologic techniques provide true diagnoses since the causative agent is demonstrated directly, differing from other more sophisticated techniques such as immunoassays. Though the high sensitivities of immunoassays are recognized, the positive and negative results obtained with them are interpreted in terms of probability. Also, it has been reported (17) that, in some instances, multiple stool specimen analyses were required to improve the diagnosis of enteroparasitosis using these assays.

There are different ways to evaluate the diagnostic performance of a technique. In the present interlaboratory evaluations we focused on some procedures with which we have become familiar considering the development and evaluation of new reagents (18), quality control analysis (19), and comparison of techniques (20) for the diagnosis of some parasitic and viral infections.

In general, the diagnostic performance of the TF-Test was better than that of conventional techniques. This finding was expected, since pooling three specimens from different days (7), or even three specimens from the same day (21), is a process that concentrates or enriches O&P. The data in Table 4 illustrate the increase in parasite yields, even with less sensitive conventional techniques, using two and three repeats. In the combination of these techniques with three repeats, the final sensitivity became as high as that of the TF-Test since 144 (41.3%) subjects with previously undetected parasites became positive. Depending on the frequency of parasitic infections and the technique used, it has been reported that three O&P examinations yield 22.7% (3) to 41.7% (4,5) additional positive results.

The evaluation of the TF-Test was performed in laboratories showing different degrees of positivity: 1) low frequency of intestinal parasitic infections (B); 2) high frequency of infections (C and D); and 3) intermediate frequency of infection (A), in an endemic zone for enteroparasitosis. In these laboratories, there were single, double, or multiple parasitic infections, with the prevalence of a higher frequency of protozoan infections than helminth infections. This profile, however, is consistent with the epidemiological data obtained over the last 10 years at different localities in the State of São Paulo (22,23).

In the second type of evaluation, we analyzed the efficiency of the techniques based on the κ index of agreement for positive and negative results in relation to the reference data. The κ index better defines the diagnostic performance of different techniques rather than providing a simple estimate in terms of the percent agreement or disagreement.

In laboratory B, although the sensitivity of the TF-Test did not significantly differ from that of the Coprotest, the κ index of the TF-Test was found to be significantly higher than that of the Coprotest. This can be explained by the fact that the κ index dealt with a large number of negative results (257) in addition to the positive results used for the evaluation of sensitivity.

The κ index of this technique was ranked as *almost perfect* for laboratories A and B and *substantial* for laboratories C and D. Possibly in the latter laboratories there were some factors influencing the efficiency of the new technique such as: 1) different concepts and procedures introduced for both patients and laboratory personnel; 2) the need of more technical skill similar to that acquired for the routinely used technique(s); and 3) variance among technicians, since more than two technicians participated in those laboratories where the frequency of multiple parasitic infections was high. However, the present findings speak in favor of the new technique.

The TF-Test proved to be flexible, providing several options for the collection of stool specimens such as: 1) the use of a desired preservative solution, or the collection of one specimen without and the other two with a preservative solution in cases in which bacterial culture is requested; 2) when staining procedures are required in the search for some coccidian oocysts in immunocompromised subjects; or 3) even to collect three specimens from the different parts of the same stool (21); etc. Also, in treated patients or in programs for enteroparasitosis control, this technique may be useful because of its high sensitivity, considering that antibody detection is ineffective for treated patients.

The TF-Test was designed to improve the parasitologic examination of stool specimens and, in this respect, the data obtained demonstrate that the objective was attained. Also, the information collected through questionnaires filled out by the users, i.e., outpatients and laboratory staff (data not shown) confirmed its useful features. The main advantages of the TF-Test are high sensitivity, suitable cost/benefit ratio, practical and easy specimen handling and processing in the laboratory, a small laboratory area required for working with it, and rapidly obtained results.

Thus, the high sensitivity and economical and practical aspects of the TF-test show that the test is applicable to individual diagnosis and epidemiological surveys. Moreover, this technique may contribute efficiently to the monitoring of chemotherapy during the follow-up of populations treated in programs of enteroparasitosis control.

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