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RESEARCH ARTICLE

Feconomics®: A Simple, Novel and Fast Technique for Stool Concentration in Parasitology Laboratory

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Summary

Feconomics® is a new ready-to-use kit for fecal concentration that eliminates the need for centrifugation and floatation by using absorbent beads. To assess its efficacy in the diagnosis of intestinal parasites, a comparative, double-blind study was conducted in the Parasitology Laboratory of Celal Bayar University Medical School. Stools (Group I, n=251) submitted for routine ova and parasite examination were concentrated with both routine formalin ethyl acetate concentration (FEAC) technique and Feconomics®. Since the number of helminthes identified in the stool samples of patients were very low, helminthes obtained from the animal models in the laboratory were included (Group II, n=11). The iodine-stained samples of all stools and some of the positive samples stained with Gomori's trichrome and Kinyoun's acid fast stain were read by specialists. In Group I, 103 of 251 (41.04%) samples were found to be positive for one or more intestinal parasites; among them, 76 (30.28%) and 96 (38.25%) stools were found to be positive with FEAC and Feconomics®, respectively, and the difference was significant (P=0.000). Same parasites were identified with both methods among all 11 samples in Group II. There was no difference between the methods for the morphological integrity and visual appearances of the parasites having cyst or egg forms; yet, it was noticed that the vegetative forms of the parasites were only identified with Feconomics®. Review of our data indicated that Feconomics® may be suggested as a fast and effective fecal concentration method for Parasitology laboratories owing to the identification of higher number of parasites compared to FEAC, and parasites with only vegetative forms such as *Dientamoeba fragilis*.

Keywords: *Diagnosis, Intestinal parasite, Concentration, Turkey*

Feconomics®: Parazitoloji Laboratuvarında Dışkının Konsantrasyonu İçin Basit, Yeni ve Hızlı Bir Yöntem

Özet

Feconomics® parazitoloji tanısı için yeni geliştirilen kullanıma hazır bir kit olup içerdiği emici boncuklar ile santrifüj ya da flotasyona gerek duyulmadan dışkının konsantre edilmesini sağlayabilmektedir. Bağırsak parazitlerinin tanısında Feconomics®'in etkililiğini değerlendirmek amacıyla, Celal Bayar Üniversitesi Tıp Fakültesi Parazitoloji Anabilim Dalı laboratuvarlarında karşılaştırmalı, çift-kör bir çalışma gerçekleştirilmiştir. Bu amaçla, laboratuvara rutin inceleme için gönderilen dışkı örnekleri (1. Grup, n=251) hem rutin uygulanan formol etil asetat konsantrasyon (FEAK) yöntemi ile hem de Feconomics® ile konsantre edilmiştir. Hasta örneklerinde saptanan helmintlerin sayısı çok az olduğundan, laboratuvardaki hayvan modellerinden elde edilen helmintler de çalışmaya dâhil edilmiştir (2. Grup, n=11). Dışkı örneklerinden hazırlanan Lugol preparatları ile bazı pozitif örneklerin trikrom ve Kinyoun preparatları uzmanlarca mikroskobik olarak değerlendirilmiştir. Yapılan incelemelerde, 1. Grup'taki 251 örneğin 103'ünün (%41.04) bir ya da birden fazla bağırsak paraziti içerdiği belirlenmiş, bunların 76 (%30.28)'sı FEAK, 96 (%38.25)'sı ise Feconomics® ile pozitif bulunmuştur. İki yöntem arasındaki fark anlamlı bulunmuştur (P=0.000). İkinci gruptaki 11 örneğin tamamında her iki yöntemle aynı parazitler tespit edilmiştir. Saptanan kist ya da yumurta formundaki parazitlerin yapısal bütünlük ya da mikroskobik görünümleri açısından iki yöntem arasında herhangi bir farklılığa rastlanmamıştır, ancak parazitlerin trofozoit formlarının sadece Feconomics® ile saptanabildiği dikkat çekmiştir. Elde edilen veriler değerlendirildiğinde, Feconomics® ile gerek FEAK'a göre daha fazla sayıda parazit saptandığından, gerekse sadece trofozoit formu bulunan *Dientamoeba fragilis* gibi parazitlerin tespit edilmesi mümkün olduğundan, Feconomics®'in parazitoloji laboratuvarları için hızlı ve etkili bir konsantrasyon kiti olarak kullanılabileceği düşünülmüştür.

Anahtar sözcükler: *Tanı, Bağırsak paraziti, Konsantrasyon, Türkiye*

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INTRODUCTION

Parasitic infections of the gastrointestinal tract are very common across the world, and gastrointestinal parasites are reported as major causes of morbidity and mortality [1]. Microscopic examination of stool specimens, normally called the ova and parasite examination, is essential in the diagnosis of intestinal parasites and consists of three separate techniques: the direct wet smear, the concentration, and the permanent stained smear. The concentration of stool samples allows the detection of parasites in low numbers in the specimen, which may be missed by using only a wet smear [2,3]. The increase in the sensitivity of microscopic examination was noted in many studies after the application of stool concentration methods [2,3].

There are two types of concentration methods, sedimentation and flotation, both of which are designed to isolate protozoa, larvae and eggs of helminthes from fecal material by centrifugation or variation in specific density of the microorganisms. Modification of the original sedimentation method of Ritchie is preferred by the majority of clinical laboratories worldwide [4,5]. The concentration procedures are labor-intensive; require relatively long time, and necessitate a specific device for centrifugation.

Owing to these drawbacks, fecal concentration devices have been commercially available since 1978, helping to standardize the concentration procedure by providing consistency in methodology [5]. Their diagnostic efficacies were found to be comparable to routine sedimentation technique [6,7].

Sodium acetate - acetic acid - formalin (SAF) solution is a common fixative that is commonly used in Parasitology laboratories. Compared to other fixative solutions, it has a relatively longer shelf-life, and an easy-to-prepare formula. It does not contain mercury compounds, which makes it relatively less toxic; additionally, the morphology of organisms in SAF-preserved stools is not as delicate as the organisms fixed in mercury-containing solutions. In addition, the sediment can be used to prepare permanent smears [2,3].

Feconomics® is a new ready-to-use kit for Parasitology laboratories and is comprised of a plastic cup containing SAF solution, and a small plastic bag containing absorbent beads of 1 to 3 mm in diameter. These small, specially-designed absorbent beads mediate the concentration of stool samples without any need for floatation or centrifugation, and the stool concentrate is ready for microscopic examination in 5 min.

The aim of the present study was to assess the efficacy of Feconomics® in the diagnosis of intestinal parasites in comparison with the routine formalin ethyl acetate concentration (FEAC) method.

MATERIAL and METHODS

A comparative, double-blind study was designed in the Parasitology Laboratory of Celal Bayar University Medical Faculty in Manisa, Turkey, to assess the efficacy of Feconomics®, in the diagnosis of intestinal parasites in comparison with routine formalin ethyl acetate concentration (FEAC) technique. Stool samples of individuals (Group I, n=251) submitted for routine ova and parasite examination were concentrated both with routine formalin ethyl acetate concentration (FEAC) technique and Feconomics®. Since very few helminthes were identified in patients' stool samples, intestinal helminthes identified with the microscopic examination of the fresh stool samples of animal models in the laboratory (such as *Trichostrongylus sp., Hymenolepis nana*) were added to the study (Group II, n=11).

The routine FEAC technique was applied as described by Garcia and Bruckner [2]. In the final step, pea-sized sediment was mixed with one drop of iodine solution on slide and this final preparation was examined at both x100 and x400 magnifications on the light microscope.

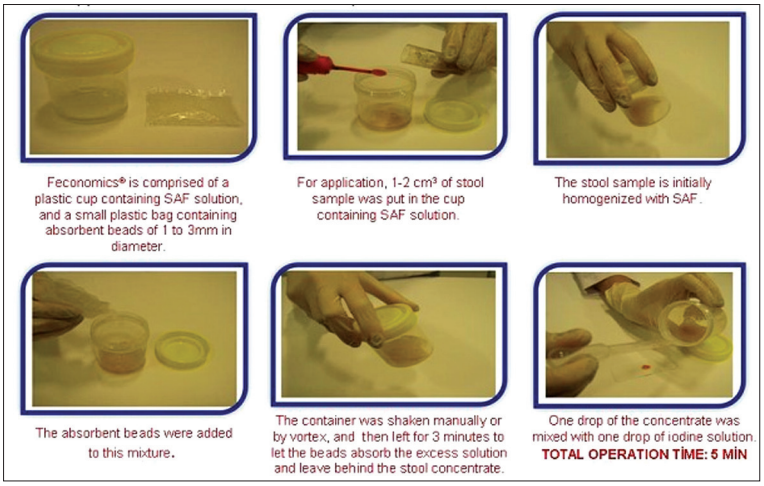

Fig 1. Application of Feconomics®

Each plastic bag in the package weighs almost 14 grams and contains approximately 800 absorbent beads, with diameters between 1-3 mm

Şekil 1. Feconomics® in uygulanışı

Plastik torbaların her biri yaklaşık 14 gram ağırlığında olup, çapları 1-3 mm arasında değişen yaklaşık 800 emici küre içermektedir

For the application of Feconomics®, 1-2 cm^3 of stool sample was put in the cup containing SAF solution, and then the absorbent beads were added to this mixture. To homogenize the mixture, the container was shaken manually or by vortex, followed by awaiting 3 min to let the beads absorb the excess solution and leave behind the stool concentrate. One drop of the concentrate was mixed with one drop of iodine solution, and examined at both x100 and x400 magnifications on the light microscope, as in FEAC technique.

To assess the visual clarity of parasites in stained smears of specimens processed by Feconomics®, at least one stool sample of each parasite were stained with Gomori's trichrome, as described by Garcia and Bruckner [2]; the total number of trichrome-stained smears examined during the study was 32. In addition, a total of 28 randomly-selected semi-form to liquid stool samples were stained with Kinyoun's acid-fast method to assess the presence of the intestinal coccidia, such as *Cryptosporidium, Cyclospora* and *Isospora* species.

The statistical analyses of the study data were conducted with SPSS® 15.0 using Pearson's chi-squared test. Taking FEAC as the golden standard, sensitivity and specificity values, and positive and negative likelihood ratios (LRs) of Feconomics® were calculated accordingly *(http://araw.mede.uic.edu/cgi-ebm/testcalc.pl)*.

RESULTS

It was noted that 43 of 251 (17.13%) of stools were in a shape between semi-form to liquid, while 148 (58.96%) were semi-form and the remaining 60 (23.90%) were solid stools. The assessments revealed that 103 of 251 (41.04%) samples in Group I were positive for intestinal parasites by at least one of the methods. *Blastocystis* was found to be the most common parasite, followed by *Giardia lamblia (Table 1)*. All samples in Group II were found to be positive for helminthes in both methods, as identified previously. Among the positive samples in Group I, 76 (30.28%) and 96 (38.25%) samples were positive with FEAC and Feconomics®, respectively, and the difference was found to be statistically significant (P=0.000) *(Table 2)*. It was also noted that 6 Feconomics®-negative samples were positive with FEAC while 26 FEAC-negative samples were positive with Feconomics®, where the difference was statistically significant, as well (P=0.000) *(Table 2)*.

Considering FEAC as the golden standard for parasitological diagnosis, the sensitivity and specificity of Feconomics® was calculated as 0.92 and 0.85, respectively. Positive and negative likelihood ratios were found as 6.20 and 0.09, respectively.

A prominent finding of the study was the identification of *Giardia lamblia* and *Dientamoeba fragilis* trophozoites in specimens processed with Feconomics®, but not with FEAC. Morphological integrity and visual appearances of these trophozoites were noted as well-preserved in Feconomics®, as well.

Examination of the Kinyoun-stained smears revealed no intestinal coccidian parasites in the present study.

DISCUSSION

Our results indicated that concentration of stool samples with Feconomics® yielded significantly more positive results

Table 1. *Number of parasites identified with FEAC* or Feconomics® during the study* (n=251)***
Tablo 1. *Çalışmada FEAK* ya da Feconomics® ile saptanan parazit sayıları (n=251)***

Identified Parasite	FEAC**	FECONOMICS®
Blastocystis	39	54
Giardia lamblia	32	32
Entamoeba histolytica/dispar	1	3
Dientamoeba fragilis	-	1
Iodamoeba bütschlii	5	4
Chilomastix mesnili	2	9
Retortomonas intestinalis	1	2
Enteromonas hominis	-	2
Endolimax nana	1	2
Entamoeba coli	3	3
Entamoeba hartmanii	-	1
Hymenolepis nana	4	4
Taenia saginata	1	1

** A total of 32 and 28 stool samples were stained with trichrome and Kinyoun's stains, respectively (Sırasıyla 32 ve 28 dışkı örneği trikrom ve Kinyoun boyaları ile boyanmışlardır)*
*** **FEAC:** Formalin ethyl acetate concentration method (**FEAK:** Formol etil asetat konsantrasyon yöntemi)*

Table 2. *Comparison of Feconomics® and Formalin Ethyl Acetate Concentration (FEAC) methods*
Tablo 2. *Feconomics® ve Formol etil asetat konsantrasyon (FEAK) yöntemlerinin karşılaştırılması*

		Formalin Ethyl Acetate Concentration (FEAC)					
FECONOMICS®		No Parasite Detected		At Least One Parasite Detected		Total	
		n	%	n	%	n	%
	No parasite detected (n, %)	149	96.13	6	3.87	155	100.00
	At least one parasite detected (n, %)	26	27.08	70	72.92	96	100.00
	Total (n, %)	175	69.72	76	30.28	251	100.00

compared to FEAC (P=0.000). Taking FEAC as the golden standard, the sensitivity and specificity of this new method were found to be relatively high. In addition, morphological integrity of the parasites, especially the trophozoites, were well-protected with Feconomics®, which is essential in the parasitological diagnosis of stool samples.

Parasitology relies less on instrumentation, automation and technological development compared to microbiology [5]. Despite the overwhelming developments in the molecular diagnosis of parasitic infections in the last decade, microscopic examination is still the first-line procedure for the diagnoses of intestinal parasites. Concentration is a crucial step for effective microscopic examination, and modified Ritchie's sedimentation method is still used predominantly in clinical laboratories all over the world, including Turkey [2,5].

In many studies, the assessments of the data revealed the necessity to use concentration methods after direct microscopy [8,9]. Similar results were reported in laboratory studies conducted in Turkey. Keskinler et al.[10] reported that the number of parasite-positive stool samples was doubled after the application of concentration methods to stool samples. Concentration methods were found to be effective, especially in the identification of parasitic cysts and/or eggs when they are in low numbers in stools [11-13]. Comparison of seven different stool concentration methods used in Parasitology laboratory revealed that the sedimentation methods were relatively more labor-intensive and helminth eggs were easier to identify compared to protozoan cysts [13]. It was also noted that none of the seven concentration methods were found to be expensive, and combination of simple sedimentation methods and floatation with zinc sulphate was suggested to improve the quality of diagnosis in routine laboratories [13].

There are many commercially-available stool concentration devices in the market, which contributed to gaining a standardized procedure, consistent methodology, and thus improved parasite recovery. Efficacies of these devices in the recovery of intestinal parasites were compared with routine FEAC method with promising results [6,7]. Perry and his colleagues compared four commercially-available stool concentration systems with FEAC method, and reported that they all concentrated stools effectively compared to direct examination [5]. Our findings yielded similar results with Feconomics®, which identified significantly more parasites than FEAC.

Clarity of sediment, lack of debris and uniformity of background material and the count of parasites in the sample are all significant in the identification of parasites in concentrated samples [3,5]. False-negative results obtained by FEAC method were generally related to low parasite counts in the stool sample [7]. Feconomics® is a new technique that produces no sediment or debris in the examined sample. It is found to be effective in the concentration of parasites with lower counts in our study groups; all 20 samples in Group I that contained one or more parasites overlooked with FEAC but identified with Feconomics®, had low parasite counts.

An interesting finding of the study was the detection of trophozoites of *Giardia lamblia* and *Dientamoeba fragilis* only with Feconomics® after staining with Gomori's trichrome. Contrary to formalin and merthiolate-iodine-formaldehyde (MIF), stool samples preserved in SAF could be stained with permanent dyes such as trichrome and hematoxylene eosin [2]. Although staining with hematoxylene eosin reveals much better visual clarity, we observed delicate trophozoites of *D. fragilis* and *G. intestinalis* in Gomori's trichrome stained smear.

Molecular diagnostic methods are applied to fresh stool samples not kept in fixatives or samples kept at -20ºC [2]. However, Troll et al.[14] reported that PCR could be conducted with SAF-preserved stool samples, even though the sensitivity declined within 2 days. Thus, it may be possible to conduct further molecular studies with the stool sample concentrated in Feconomics®, which warrants further assessments.

Feconomics® is an effective new tool in the concentration of stool samples in parasitological diagnosis. It obviates the need for centrifugation and creates no debris after the procedure is completed. It is highly effective in the identification of parasites in specimens with lower counts, and maintains the morphological integrity of the parasites.

In addition, the whole procedure takes 5 min, which is a distinct advantage as other concentration methods were found to take 7 to 50 minutes in a recent study [13]. Indeed, it may be less hazardous for the laboratory staff compared to routine FEAC method. Owing to all these advantages, Feconomics® may be recommended for routine diagnosis of intestinal parasitic infections.

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