

FLOTAC Technique for Soil-Transmitted Helminth Infection Diagnosis

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Abstract

Human helminthiasis is a neglected disease with significant economic impacts caused by its effects on performance and cognition. The burden of many helminth infections is not well understood due to the lack of progress in detecting low-intensity infection in elimination programs. Furthermore, the decision for individual and community treatments, as well as the community-based control program evaluation, obviously depends on the technics used for parasitological diagnostic. A well-established diagnostic technic will be beneficial to detect and eliminate the disease. Therefore, this study aimed to compare the performance of FLOTAC and FECT technics for detecting helminth infections in human stool. A total of 149 fecal specimens were collected from schoolchildren in Nangapanda village, Ende District, East Nusa Tenggara Province in 2012. The sensitivity of both technics was analyzed using the kappa analysis. Positive results from both technics were used as the gold standard. The sensitivity of FLOTAC for diagnosing *T. Trichiura*, *A. lumbricoides* and hookworm infections were 100%, 100%, and 82%, respectively, while the sensitivity of FECT was 80%, 7%, and 18%, respectively. FLOTAC yielded considerably higher mean faecal egg counts (11,452, 1,038, and 19 eggs per gram stool (EPG) for *A. lumbricoides*, *T. Trichiura*, and hookworm). FLOTAC technique was considerably more sensitive than FECT in diagnosing soil-transmitted helminth infections. In conclusion, FLOTAC can be used as a diagnosis tool for future helminth control programs.

Keywords: Diagnosis, FLOTAC, soil-transmitted helminth

Penggunaan Teknik FLOTAC pada Diagnosis Infeksi Cacing Usus

Abstrak

Cacingan termasuk salah penyakit yang terabaikan dan masalah kesehatan masyarakat yang berdampak signifikan terhadap ekonomi, yaitu melalui kinerja dan kognitif. Sementara morbiditas cacingan sering tidak dipahami dengan baik dalam program eliminasi karena terbatasnya sensitivitas deteksi infeksi pada daerah yang intensitas infeksi rendah. Selain itu, akurasi teknik diagnostik parasitologi penting dalam penentuan pengobatan individu dan masyarakat serta evaluasi program pengendalian melalui pengobatan pada komunitas (masyarakat). Penelitian ini membandingkan dua teknik, yaitu FLOTAC dan FECT dalam mendiagnosis infeksi cacing pada sampel tinja manusia. Sebanyak 149 spesimen feses dikumpulkan dari anak-anak sekolah di Nangapanda, Ende Kabupaten, Provinsi Nusa Tenggara Timur tahun 2012. Sensitivitas dari kedua teknik tersebut diukur menggunakan analisis Kappa. Hasil positif pada kedua teknik atau hasil kombinasi yang digunakan sebagai gold standar. Sensitivitas FLOTAC dalam mendiagnosis *T. trichiura*, *A. lumbricoides* dan cacing tambang adalah masing-masing 100%, 99% dan 82%, sedangkan sensitivitas FECT adalah 82%, 75% dan 18%. Teknik FLOTAC memperoleh rata-rata *faecal egg count* (11.452, 1038, 19 telur per gram tinja (EPG) untuk *A. lumbricoides*, *T. trichiura*, dan cacing tambang. Teknik FLOTAC lebih sensitif dibandingkan teknik FECT untuk mendiagnosa infeksi cacing usus. Simpulan, FLOTAC sangat mungkin menjadi alat diagnosis untuk program pengendalian cacing di masa depan.

Kata kunci: Diagnosis, FLOTAC, infeksi cacing usus

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Introduction

Helminthic parasites afflict more than a quarter of the world's population, causing significant disease and disability. *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm (*Ancylostoma duodenale* and *Necator americanus*) are known as soil-transmitted helminthiasis because they require soil for the development of the infective form¹

According to Montesor et al,² preventive chemotherapy (PC) implemented between 2010 and 2015 saved over 549 000 DALYs in 2015, which is equivalent to 44 % of the disease burden (in DALYs) in 2010 prior to global STH control program expansion.

Helminthiasis is a major public health concern in Indonesia. A cross-sectional survey of 16 villages was conducted in Semarang demonstrate that STH overall prevalence was 33.8%.³

The primary method of preventing infection STH is treated on a regular basis with a four-drug regimen recommended by WHO, namely mebendazol (MBD), albendazole (ALB), levamisole, and pyrantel.⁴

Anthelmintic Cure Rate (CR) and Egg Reduce Rate (ERR) were two indicators that were used to determine therapeutic effectiveness in individuals (ERR). The ERR indicator is preferred in public health. Although the WHO recommends parasitological examinations every few years for large-scale treatment programs, this is rarely done, and thus often occurs in the absence of a coherent long-term strategy and standard monitoring protocol.⁵ When infection levels are typically highest, a diagnostic test with moderate sensitivity is acceptable; however, the chosen tool must be simple to use, cost-effective, and capable of testing large populations in a high-throughput manner.⁶

The most common method of diagnosis is to calculate the EER using the Kato-Katz and concentration techniques. However, the Kato-Katz method of calculating the EPG has limitations for use in the field to monitor the STH elimination program, particularly because stool samples must be read within 30–60 minutes of collection to avoid hookworm egg damage. The concentration method can be used to calculate the density of infection in this manner, but the calculations are less sensitive than the Kato-Katz technique, especially when the density of eggs is low (4–5 eggs)⁷

According to WHO, if the number of eggs in stool specimens remains low, direct examination is not recommended. Concentration techniques

are required when direct examination fails to find the parasite despite the patients exhibiting clinical symptoms of parasitic infections and to detect the worm *Schistosoma* and *Taenia* infection. The WHO recommended concentration technique is formalin formalin ether or ethyl acetate (FECT). The FECT was more effective than other methods in detecting hookworm, *Trichuris trichiura*, and small liver flukes.⁸

An Italy researcher have developed a new technique which is called FLOTAC to diagnosis helminth infection. FLOTAC promising diagnosis technique is sensitive, accurate and appropriate for qualitative and quantitative analysis. FLOTAC method utilizes apparatus, a cylindrical device with two 5 mL flotation chamber, which allows up to 1 g of feces to be prepared for microscopic analysis. Compared to current diagnostic methods more widely used to detect human parasites such as Kato-Katz and concentration techniques. Techniques FLOTAC showed a higher sensitivity and accuracy. All FLOTAC technique can be performed on fresh stool and stool samples preserved, and takes about 12–15 minutes of preparation time prior to review by microscopis.⁹

The sensitivities of a single Kato-Katz for diagnosing of *T. trichiura*, *A. lumbricoides* and hookworm infections were 76.6%, 67.8% and 19.6%, respectively, while the sensitivity of FLOTAC was 100% for all the three soil-transmitted helminth species. While *T. trichiura*, *A. lumbricoides*, and hookworm infections were found in 44.6 % (121/271), 21.7 % (59/271), and 3.7 % (10/271) of a single Kato-Katz technique. Kappa values of 0.74, 0.73, and 0.28 were found for *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm, respectively. For each of the three soil-transmitted helminths, a single FLOTAC showed significantly more sensitive than a single Kato-Katz.¹⁰

Filariasis and soil-transmitted helminthiasis programs, such as mass drug administration are now being implemented as part of the Indonesian National elimination. As a result, the sensitive diagnostic technique must be employed in order to support the elimination program's success. Therefore, in this study, we will compare the concentration technique, namely FECT with FLOTAC, in order to assess its sensitivity and ability to identify people who are not infected with STH infection or negative predictive values, as well as egg intensity in elementary school children where STH is endemic.

Methods

The research was carried out in Ende's Nangapanda District, East Nusa Tenggara Province in 2012. To assess sensitivity and negative predictive value (NPV), samples were obtained by calculating the proportion of the number of single subjects. The study included 149 schoolchildren from low-income communities where intestinal worms were prevalent. The sample size was calculated using the formula for a single proportion, namely the total number of subjects to assess sensitivity or specificity using disease prevalence as a reference.¹¹

$$n = \frac{Z\alpha^2 PQ}{d^2}$$

where n is Total Sample, p is Sensitivity for FLOTAC technique = 80%, Q is 10%, d is deviation of 10%, $Z\alpha$ is If the 95% confidence interval, =0.05 then $Z\alpha=1.96$, So: $n=(1,96^2 \times 0,90 \times 0,1)/0,1^2$
 $n=(3,8146 \times 0,09)/0,01$, $n=30,51 \sim 31$

Sample size calculation for sensitivity and specificity analysis for prevalence of disease from 65% in Ende Regency, East Nusa Tenggara Province is 65%, so the number of subjects is $100/65 \times 31 = 48$. A sample size of at least 48 people was required for this study.

Stool samples were sent to the Parasitology Laboratory at the Faculty of Medicine, University of Indonesia, in Jakarta. Each sample was placed in a small tube containing 10 mL of sodium acetate-acetic acid-formalin (SAF) solution. The SAF fixed sample processed by an ether concentration and FLOTAC technique was largely as described by Cringoli. The reagent or flotation solution (FS) used in this study is saturated sodium chloride (NaCl).⁹

The FS chosen has been determined by an element of the parasite that is relevant to the purpose of the study. NaCl is dissolved in 1 liter of 40–50°C warm water until all of the salt has dissolved (500 g) and the rest has settled to the bottom of the container. SODIUM NITRATE is dissolved in water with the help of a magnetic stirrer. To ensure that solution is fully saturated, leave it at room temperature overnight. With a hydrometer, measure the saturated solution's s.g. (1.2), noting that the s.g. of the saturated solution will vary slightly depending on the ambient temperature. For at least a month, maintain the flotation solution at room temperature (20–25 °C). We need to weigh 10 g of feces before

homogenizing it with 90 mL of tap water. The suspension was filtered through a wire mesh screen with a 250 µm aperture, and tubes (15 mL) were filled to 10 mL with the filtrate. The supernatant was removed after centrifugation at 1500 rpm for 3 minutes, and the tubes were refilled to 6 mL with the appropriate flotation solution. One chamber of a FLOTAC was filled with the homogenized solution after vortex mixing the sample (5 mL). After centrifugation at 1000 rpm for 5 minutes, the FLOTAC was rotated and parasite elements were counted at 100 magnification. To ensure that the chambers were completely full and to avoid small air bubbles, a 6-mL volume has been used rather than the suggested 5-mL volume.⁹

Data was entered into Microsoft excel Office 2007 and analyzed with SPSS software for Windows version 17. The sensitivity and NPV were calculated using the CAT maker software developed by Douglas Badenoch of the Centre for Evidence-Based Medicine (CEBM, University of Oxford, England). The CAT maker software is a tool that assists us in creating Critically Appraised Topics, or CATs, for key articles we encounter about therapy, diagnosis, prognosis, aetiology/harm, and systematic reviews of therapy. The CAT maker software as the toolkit adds to the arsenal of critical appraisal tools currently available and is especially useful in assessing evidence from a wide variety of research design.¹²

The statistic was used to compare the agreement between the two techniques for diagnosing STH infection (FECT versus FLOTAC). The sensitivity (the proportion of true positives in the population) and negative predictive value (the proportion of individuals with a negative test result who are correctly diagnosed) of each method have been calculated, with the combined results from each technique serving as the diagnostic "gold" standard. This method maximized sensitivity values through detecting STH (*A.lumbricoides*, *T.trichiura*, *hookworm*) eggs using any of the techniques used.

Ethics approval was obtained from the Faculty of Medicine University of Indonesia's research ethics committee (reference no. 194/PT02.FK/Etik/2006). The study was ethically approved by The study's purpose and procedure were clearly explained to selected samples (school-aged children, and the faecal samples have been provided by children who agreed to provide their stools). Furthermore, at the end of this study, all samples (school-aged children), including those who tested positive for helminth infection, were given antihelminthic drug albendazole 400 mg.

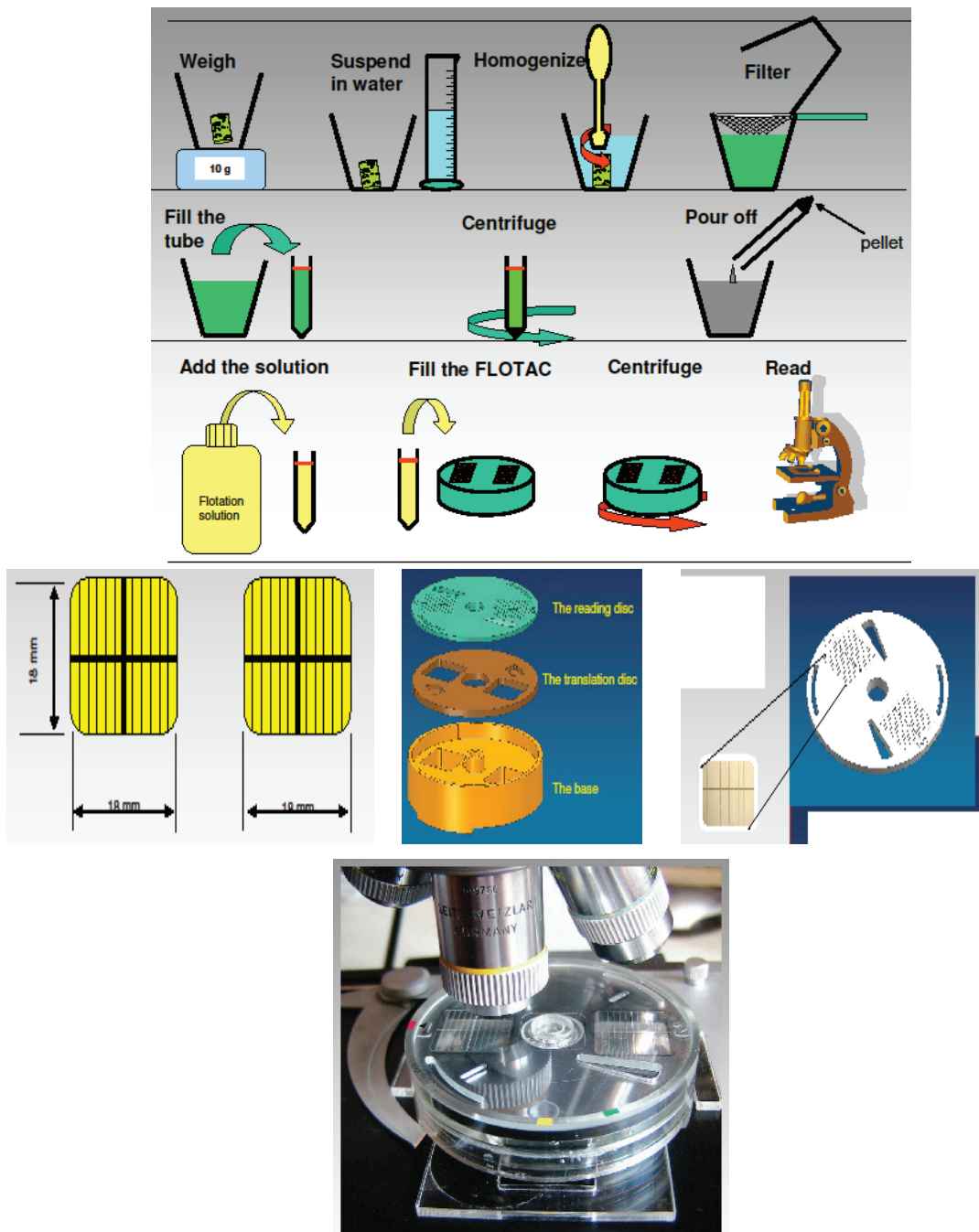


Figure 1 FLOTAC Technique Procedure¹³

Results

The research was conducted in four elementary schools which included 149 school-age children and the analysis shows the variation of prevalence rates ranging between 37.14–62.69%. SD Pau Pau has the highest prevalence of 62.69%. This study revealed that 78 (57.68%)

boys and 71 (49.30%) girls providing their stool. The examination technique (FECT) is 53% (79/149), and the FLOTAC examination is 64.4 % (96/149), shows a high morbidity rate for STH parasites. However, it cannot be distinguished from the risk of disease infection in both boys and girls. The incidence of worm infection by age group was divided by class (I to VI) in that study,

Table 1 Distribution of Helminthiasis Cases And The Causative Species By Gender, Age

Characteristic	No.of Sample Examined (n=149)	No. of Positive	%age (%)	Infection by STH species		
				Al	Tt	Ct
Sex						
Male	78	45	57.69	23	39	2
Female	71	35	49.30	19	33	0
School grade						
Grade I	23	12	52.17	7	10	1
Grade II	34	18	52.94	9	16	1
Grade III	26	16	61.54	6	15	0
Grade IV	16	8	50.00	5	7	0
Grade V	18	8	44.44	5	7	0
Grade VI	32	18	56.25	10	17	0
Areas of School						
SD Katolik Arnoldus	35	13	37.14	7	11	0
SD Negeri Nangapanda2	31	16	51.61	11	15	0
SD Negeri Puu'Au	16	9	56.25	6	18	1
SD Negeri Puu'Pau	67	42	62.69	19	30	1

with results ranging from 50% to 76.92%. As a result, nearly every grade level had an infection rate greater than 50%.

FLOTAC detected 51 of 149 samples, while FECT detected 42 *A. lumbricoides* cases. Both of the above techniques appear to have high agreement attributes in identifying the species *A. lumbricoides* (p=0.73). The FECT technique detects 72 cases of *T.trichiura*, while the FLOTAC

technique detects 91 cases. The agreement between the two methods for detecting *T.trichiura* appears to be high (p=0.86). The FECT technique, on the other hand, detects only two cases of hookworm, whereas FLOTAC detects nine cases. In both techniques, positive hookworm species were not always found to be the same for both techniques. The agreement between the two techniques in detecting

Table 2 *A.lumbricoides, T.trichiura, Hookworm Infection Status Diagnosing with FECT and FLOTAC Technique*

Species	FECT	FLOTAC			McNemar Test CI 95%	k agreement (p)
		Positive	Negative	Total		
<i>A. lumbricoides</i>	Positive	42	0	42	0.001	0.72(<0.001)
	Negative	9	98	107		
	Total	51	98	149		
<i>T. Trichiura</i>	Positive	71	1	72	0.065	0.86(<0.001)
	Negative	20	57	77		
	Total	89	58	149		
Hookworm	Positive	0	2	2	0.004	-0.022(<0.001)
	Negative	9	138	147		
	Total	9	140	149		
Any infection*	Positive	80	0	80	0.001	0.76(<0.001)
	Negative	17	52	70		
	Total	97	52	149		

CI=confidence interval; p<0.05 with McNemar Test *Individuals infected with at least one of the species

Table 3 Sensitivity and NPV of the FLOTAC and FECT Technique

Species	Sensitivity(95%CI)		NPV (95%CI)	
	FLOTAC	FECT	FLOTAC	FECT
<i>A.lumbricoides</i>	100% (100-100%)	82% (72-93%)	100%(100-100)	92%(86-97%)
<i>T.Trichiura</i>	99% (97-100%)	78% (70-87%)	98%(95-100%)	75%(65-84%)
Hookworm	82% (59-100%)	18% (--5-41%)	99(9 -100%)	94%(90-98%)
Any infection*	100% (100-100%)	82% (75-90%)	100%(100-100%)	75%(65-86%)

* Individuals infected with at least one of the species

Table 5 Variation in the Density of *A. lumbricoides*, *T. trichiura*, and Hookworm Infection Using FECT and FLOTAC Techniques

Species	FECT	FLOTAC
<i>A.lumbricoides</i>		
No infection	108	99
Light (1-4999 epg)	41	47
moderate (5000-49999 epg)	0	3
Total	149	149
<i>Ttrichiura</i>		
No infection	77	60
Light (1-999 epg)	72	88
moderate (1000-9999 epg)	0	1
Total	149	149
Hookworm		
No infection	147	140
Light (1-1999 epg)	2	9
moderate 2000-3999 epg)	0	0
Total	149	149

negative hookworm species, on the other hand, was significant (p= -0022). The FECT technique detected 80 cases of infection, while FLOTAC detected 97 positives. Understanding these two detection methods has a high value (p= 0.76).

The FLOTAC and FECT were calculated in this study under the assumption that positive results from both techniques (combination) were considered to be “true prevalence.” However, with this approach, specificity and PPV cannot be obtained. Because the combination was considered the “gold standard,” an analysis was performed using a soft CAT maker to determine the sensitivity and NPV of both techniques.

The FLOTAC technique showed significantly higher in sensitivity and NPV compared with the FECT. Moderate sensitivity in diagnosing *Ttrichiura* (78%) and hookworm (18%) was found in the FECT technique. Moderate NPV values are found for diagnosing *Ttrichiura* species (75%) and any infection (75%). The sensitivity of FECT for diagnosing hookworm species is very low (18%).

According to the table above, the geometric mean of infection intensity among schoolchildren is 751 EPG for *A. lumbricoides*, 596 EPG for *Ttrichiura*, and 3 EPG for hookworm, as determined by FECT techniques. However, using FLOTAC techniques, the species *A. lumbricoides*, *Ttrichiura*, and hookworm have 11452EPG, 1038 EPG, and 19 EPG, respectively.

In table 5, almost all infected children were classified as having a mild infection. There are some cases where FECT does not detect light infections. The FLOTAC technique can detect a few specific infections caused by *A. lumbricoides* and *Ttrichiura* that are classified as moderate. All these techniques could only identify hookworm light infection in children.

FLOTAC had significantly higher sensitivity

Table 6 Variation of Infection Intensity by Species (*A.lumbricoides*, *T.trichiura*, hookworm) between FECT and FLOTAC Technique

Species	(95% CI)	Technique	
		FECT	FLOTAC
A. lumbricoides	Sensitivity	82%(71-92%)	100%(100-100%)
	Specitivity	100%(100-100%)	100%(100-100%)
	NPV	92%(86-97%)	100%(100-100%)
	PPV	100%(100-100%)	100%(100-100%)
T.Trichiura	Sensitivity	78%(76-86%)	99%(97-100%)
	Specitivity	100%(100-100%)	100%(100-100%)
	NPV	75%(65-84%)	98%(95-100%)
	PPV	100%(100-100%)	100%(100-100%)
Hookworm	Sensitivity	18%(5-41%)	82%(75-90%)
	Specitivity	100%(100-100%)	100%(100-100%)
	NPV	94%(90-98%)	99%(97-100%)
	PPV	100%(100-100%)	100%(100-100%)
Any infection*	Sensitivity	82%(75-90%)	99%(97-100%)
	Specitivity	100%(100-100%)	100%(100-100%)
	NPV	76%(66-86%)	98%(94 -100%)
	PPV	100%(100-100%)	100%(100-100%)

* Individuals infected with at least one of the species

and NPV than FECT. The FECT technique had a moderate sensitivity throughout, it was able to identify *T. trichiura* (78%) and hookworm (78%) (78 percent) (18%). The NPV for diagnose *T. trichiura* species was 75% and any infection was moderate (76.5%). FECT has a very low sensitivity for identifying hookworm species.

Discussion

The current era's "preventive chemotherapy," particularly for STH human infections, has a tendency to ignore diagnostic aspects. Furthermore, STH infection has the potential to cause significant the public health burden. In terms of elimination in various epidemiological settings, an accurate diagnosis is required. It is also an essential component of proper patient management, as well as the design, implementation, and monitoring of community-based control programs. Our research findings support and validate the use of existing techniques for the in vivo diagnosis of *A.lumbricoides*, *T.trichiura*, and hookworm. The benefits and drawbacks of the comparing technique are also discussed.

The results of both formalin ethyl acetate concentration (FECT) and FLOTAC techniques show that the morbidity of STH infection among children in Nangapanda village is still high, with (FECT) at 53% (79/149) and FLOTAC at 64.4 % (96/149). However, there is no difference in risk for infection between boys and girls. Incidence of STH case infection by age was divided by grade class (I-VI), with results ranging from 50% to 76.92%. In almost every class, the infection rate was greater than 50%. The highest infection rate was found in the third class of children aged 8 years, with a prevalence of 76.92%.

The FECT technique is not more sensitive for detecting hookworms than *A. lumbricoides* and *T. trichiura* based on research and the literature Furthermore, as per studies by Steffanie et al., FECT uses ether, which can impact intestinal worm eggs. The low sensitivity of FLOTAC in detecting hookworms may be due to the fact that the sample feces are stored in 5 % formalin, which can affect the condition of hookworm eggs. The decreased sensitivity of FLOTAC in samples stored for more than 6 months in sodium acetate acetic acid-formalin (SAF). In his study, samples stored for less than 6 months using different flotation solutions (FS) and ether in the

washing phase had a positive effect on detecting worm. What is suitable for one parasite may not be suitable for another. The results obtained at this time cannot be generalized, but there is a need for optimization of the FLOTAC technique, particularly for samples stored for a long time, so that the reliability and validity are required to identify hookworms in particular. The use of formalin for preservation for more than 6 months can affect or damage hookworm eggs.¹⁴

Observations in the field revealed that schools are located in areas far from health care (PHC), and students still do not wear footwear (bare foot), and students must remove footwear during learning activities in the class, which is mandatory for all school tenants.

The majority of Nangapanda's population is low-income. *T. trichiura* infection appears to be the most common among school-aged children (70), followed by *A.lumbricoides* infection (41) and hookworm infection (2). These findings indicate that helminthiasis is still a public health concern in the Ende District, and that it requires significant attention both diagnostically and controllably. As a result, using the current WHO-recommended strategy, intestinal worm infection, also known as Neglected Tropical Disease (NTD), can be effectively eliminated and prevented.

FLOTAC had a sensitivity of 100%, 98 %, and 82 % for diagnosing *T. Trichiura*, *A. lumbricoides*, and hookworm infections, respectively, whereas FECT had a sensitivity of 82%, 75%, and 18%. Within those three scenarios, the NPV of FLOTAC is extremely high. Throughout terms of diagnosing hookworm, FECT has a very low sensitivity. FLOTAC had significantly higher mean faecal egg counts (11452, 1038,19 eggs per gram stool (EPG) for *A. lumbricoides*, *T. Trichiura*, and hookworm). When it came to diagnosing soil-transmitted helminth infections, the FLOTAC technique was far more sensitive than FECT. Finally, FLOTAC has the potential to be used as a diagnostic tool in future helminth control programs. We use the "CATmaker" analytics to predict the sensitivity and specificity of the FLOTAC and FECT) techniques. This software can be used to create Critically Approved Topics, therapies, diagnosis, prognosis, aetiology/harm, and a systematic review of therapy. Positive results from both techniques are regarded the "gold standard" and the true prevalence. Specificity and positive predictive value (PPV) may be impossible to achieve with this.¹²

These findings were analyzed using CATmaker software, specifically the diagnosis

of STH infection with the FLOTAC technique on all positive and negative samples, regardless of species, with a sensitivity of 100 % (100–100, 95 % CI) and an NPV of 100 % (100–100, CI95 %). All species of STH worms were examined. While the FECT technique has an 82 % sensitivity and a 75% NPV in diagnosing any infection. This is consistent with the findings of Kassahun Habtamu et al. in Ethiopia, who discovered that FLOTAC has a sensitivity of 100% (97.5–100, 95% CI) and an NPV of FLOTAC techniques were found to be more sensitive than multiple Kato-Katz thick smears for the diagnosis of hookworms, *A. lumbricoides*, and *T. trichiura* infections. one hundred % (94.1-100, 95 % CI).¹⁰ When compared to multiple Kato-Katz thick smears for the diagnosis of hookworms, *A. lumbricoides*, and *T. trichiura* infections, Ngwese et al reported that FLOTAC techniques were more sensitive.⁹

Nikolay et.al found that the FLOTAC method had the higher specificity at low intensity, at 81.8 % (95% BCI (Bayesian Credible Intervals): 65.5–90.3%), compared to 97.1% (95% BCI: 93.1–99.7%) at high intensity.⁷

Based on WHO criteria, almost all of the children were classified as having mild infections. According to the FLOTAC technique, three (5.8 %) of people had moderate infections (5000–49999) of *A. lumbricoides*, while 47 (94%) had mild infections (1–4999 EPG). FLOTAC classified one *T. trichiura* infection as moderate (1000–9999 EPG) and 90 (98.8 %) as mild (1–999 EPG). FLOTAC identified 9 cases (5.96 %) of mild infections (1–1999 EPG). The FECT technique detects only 41 (36.52 %) of people with a mild infection (1–4999) for the species *A. lumbricoides* and *T.trichiura* 71 (61.73%) of people with a mild infection (1-999 EPG), and 2 (1.73 %) of people with a severe infection (1–4999).

The sensitivity and specificity of the FLOTAC examination technique for worm infections, particularly *A. lumbricoides* and *T. trichiura* species, to the formalin ether or formalin ethyl acetate (FEC) examination technique is quite high, with >90% for any infection. In contrast, sensitivity was rated low for hookworm species due to the short duration of the study. FLOTAC rates EPG as very sensitive in terms of sensitivity. Furthermore, the FLOTAC technique has a high NPV, which is useful for assessing therapeutic effectiveness and patient management.

Given the high cost of collecting stool samples in the field and then analyzing them in the laboratory, WHO recommends mass treatment as a preventive measure for high-risk groups in endemic areas. In endemic areas, where

morbidity control has been shifting to infection and transmission control, an assessment of the epidemiological situation is required, and diagnosis is critical. Given the high operational costs of the helminthiasis prevalence survey, the elimination of intestinal worms through mass treatment is the most likely option for Indonesia. However, this option necessitates the use of a sensitive diagnostic technique. Currently, the molecular biology examination technique, PCR, has proven to be very sensitive, but it is not applicable on a large scale and is prohibitively expensive.⁹

Concentration and Kato Katz techniques have been proposed for detecting STH infection. Fresh faecal samples, on the other hand, must be examined immediately using the Kato Katz method because they can affect egg condition and examination results, particularly for hookworm species. The FLOTAC technique does not necessitate immediate examination because the samples are preserved with SAF or formalin, The FLOTAC technique is ideal for large sample sizes and difficult survey areas. Furthermore, stool samples preserved for up to 83 days can be used.⁹

In terms of cost, Kato-Katz method is less expensive than the Mini-FLOTAC (US\$10.14 vs. US\$13.11 for school-based sampling and US\$11.99 vs. US\$14.96 for community sampling) which is similar with Zanzibar study the unit cost for a single Kato Katz technique was \$1.73, the dual Kato Katz technique was \$2.05, and the FLOTAC technique was \$2.35.¹⁵

The FLOTAC method is more expensive than Kato Katz because the preparation and reading stages are longer, but FLOTAC has a higher sensitivity. As a result, the cost is higher, but the results are more promising.

In conclusion, FLOTAC technique has high negative predictive value (NPV) and accuracy in diagnosing STH infection (*A.lumbricoides*, *T.trichiura* and hookworm). This FLOTAC technique could be used to investigate STH infection and assess the effectiveness of deworming programs.

Finally, the FLOTAC technique has a high negative predictive value (NPV) and accuracy when it comes to diagnosing STH infection (*A.lumbricoides*, *T.trichiura* and hookworm). This FLOTAC technique could be used to diagnose STH infection and evaluate deworming program effectiveness.

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References

1. Jourdan PM, Lamberton PHL, Fenwick A, Addiss DG. Soil-transmitted helminth infections. *Lancet*. 2018;391(10117):252–65.
2. Montresor A, Trouleau W, Mupfasoni D, Bangert M, Joseph SA, Mikhailov A, et al. Preventive chemotherapy to control soil-transmitted helminthiasis averted more than 500 000 DALYs in 2015. *Trans R Soc Trop Med Hyg*. 2017;111(10):457–63.
3. Kurscheid J, Laksono B, Park MJ, Clements ACA, Sadler R, McCarthy JS, et al. Epidemiology of soil-transmitted helminth infections in semarang, central java, indonesia. *PLoS Negl Trop Dis*. 2020;14(12):1–17.
4. Moser W, Schindler C, Keiser J. Efficacy of recommended drugs against soil transmitted helminths: Systematic review and network meta-analysis. *BMJ*. 2017;358:1–10.
5. WHO. Assessing The Efficacy Of Anthelmintic Drugs Against Schistosomiasis And Soil-Transmitted Helminthiasis. Vol. 148. 2013. 1–38 p.
6. Solomon AW, Engels D, Bailey RL, Blake IM, Brooker S, Chen JX, et al. A diagnostics platform for the integrated mapping, monitoring, and surveillance of neglected tropical diseases: Rationale and target product profiles. *PLoS Negl Trop Dis*. 2012;6(7):1–5.
7. Nikolay B, Brooker SJ, Pullan RL. Sensitivity of diagnostic tests for human soil-transmitted helminth infections: A meta-analysis in the absence of a true gold standard. *Int J Parasitol*. 2014;44(11):765–74.
8. Brummaier T, Archasuksan L, Watthanakulpanich D, Paris DH, Utzinger J, McGready R, et al. Improved detection of intestinal helminth infections with a formalin ethyl-acetate-based concentration technique compared to a crude formalin concentration technique. *Trop Med Infect Dis*. 2021;6(2).
9. Ngwese MM, Manouana GP, Moure PAN, Ramharter M, Esen M, Adégnika AA. Diagnostic techniques of soil-transmitted

- helminths: Impact on control measures. *Trop Med Infect Dis.* 2020;5(2):1-8.
10. Habtamu K, Degarege A, Ye-Ebiyo Y, Erko B. Comparison of the Kato-Katz and FLOTAC techniques for the diagnosis of soil-transmitted helminth infections. *Parasitol Int.* 2011;60(4):398-402.
 11. Bujang MA, Adnan TH. Requirements for minimum sample size for sensitivity and specificity analysis. *J Clin Diagnostic Res.* 2016;10(10):YE01-6.
 12. Moralejo D, Ogunremi T, Dunn K. Critical Appraisal Toolkit (CAT) for assessing multiple types of evidence. *Can Commun Dis Rep.* 2017;43:176-81.
 13. Cringoli G. FLOTAC, a novel apparatus for a multivalent faecal egg count technique. *Parassitologia.* 2006;48(3):381-4.
 14. Knopp S. Diagnosis, epidemiology and control of soil-transmitted helminth infections in Zanzibar , Tanzania. In University of Basel; 2011. p. 1-45. Available from: http://edoc.unibas.ch/diss/DissB_9646
 15. Assefa LM, Crellen T, Kepha S, Kihara JH, Njenga SM, Pullan RL, et al. Diagnostic accuracy and cost-effectiveness of alternative methods for detection of soil-transmitted helminths in a post-treatment setting in Western Kenya. *PLoS Negl Trop Dis.* 2014;8(5):e2843.