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Development of Modified Kato Katz Kit as Laboratory Scale Prototype for Early Detection of Soil-Transmitted Helminths (STHs) Infection

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Abstract: Early detection of Soil-Transmitted Helminths (STHs) infection in laboratory medical facilities is one of the government's efforts in its elimination. Government regulations recommend Kato-Katz's quantitative method for the diagnosis of STHs infection. Kato-Katz's domestic product is not available yet. This research aims to create a laboratory-scale modified Kato-Katz kit for early detection of STHs infection by fecal egg counts (FECs) in estimating the number of eggs per gram of stool (EPG). An experimental study was conducted to create a resin template, spatulas, and 3M-adhesive tape as a modified Kato-Katz Kit. 120 Kato-Katz thick smear preparations were done from 30 samples for modified and standard Kato-Katz with duplication. The amount of stool for each was weighed. All Kato-Katz thick smear microscopic examination was done at least 60 minutes by identifying the STHs eggs and counting them. The result for both methods showed no differences in EPG for Trichuris trichiura eggs p-value= 0.012 ($p<0.05$), meanwhile the differences for Ascaris lumbricoides eggs p-value= 0.156, and Hookworm eggs p-value=0.102 ($p>0.05$). Under microscopic examination, some A. lumbricoides eggs morphology was not visible because the adhesive in modified Kato-Katz blurry it, and the Hookworm fragile eggshells made it disappear within less than 60 minutes. The study concluded no difference in EPG for T.trichiura eggs by FECs between modified Kato-Katz as this study product with the standard. It is recommended to improve modified Kato-Katz in further study, especially the adhesive tape, before using it as a quantitative tool for early detection of STHs infection.
Keyword: Soil-transmitted helminths infection; kato-katz kit; egg count

INTRODUCTION

Worms infection, especially Soil-Transmitted Helminths (STHs), is usually clinically asymptomatic, though non-specific gastrointestinal symptoms and eosinophilia may be seen. However, over a billion people worldwide are at risk of STHs infection, i.e., *Ascaris lumbricoides, Hookworm* (*Ancylostoma duodenale and Necator americanus*), and *Trichuris trichiura*. The global burden of STHs Infection in 2017 was estimated at 1.9 million disability-adjusted life years (DALYs). (WHO, 2017; Pabalan, 2018; Jourdan, et al, 2018; Kyu, et al, 2018).

Field-survey data on STHs prevalence, infection intensity, and drug efficacy is necessary to guide the implementation of control programs worldwide and should be of the best possible quality (Ganguly, 2017). In Indonesia, early detection in the laboratory medical facilities is one of the government's efforts in the Acceleration program of Prevention and Decrease of Stunting contained in PMK No.15 of 2017 on

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Countermeasures of worms infection. The PMK is a reference for implementing early detection of infection by recommending Kato Katz as a quantitative method to define infection intensity categories in the field survey (Kemenkes, 2017).

Panggabean's (2016) study showed significant results between Kato Katz's technique with direct wet preparations, sedimentation formol ether concentration (FEC), and floatation of saturated zinc sulfate. The Kato Katz result was better than other concentration methods.

Conventional microscopy-based methods such as direct Kato–Katz smear or mounts after stool centrifugation/flotation-based concentration techniques have been the mainstay of diagnosis, especially in resource-poor countries where these infections abound. However, recently, these are being adapted to closed, easy to perform, digital formats, thereby improving the sensitivity and applicability in a remote, resource-limited setting. Kato Katz's method is the gold standard of quantitative worm eggs counts in the stool. WHO recommends a thick preparation technique of stool specimens to diagnose infection intensity at the community level (Elgendy et al., 2016; Liu et al., 2017; Calvopina et al., 2018, Khurana et al., 2021).

The technique is considered specific and straightforward, with microscopic examination of fresh specimens (41.7 mg) with a minimum of 20 eggs per gram of feces. Kato Katz technique is considered relatively cheaper (US$ 0.1 – 0.3 per Kato Katz tool kit) and easy to use for research or examination in the field (Turner et al., 2017; WHO, 2019).

On the other hand, the lack of availability of Kato Katz Kit following WHO recommendations in Indonesia initiated an imported product with a high-cost burden. Data from the Ministry of Health of Indonesia shows the value of the 2018 STATE BUDGET ceiling for the procurement of Kato Katz tool kits of 3.31 Billion while there is no robust and reliable domestically produced Kato Katz tool kit. The price of the Kato Katz kit in Indonesia is still relatively high, around Rp.10,000 - Rp.30,000 per examination. Some technicians and researchers get around using cheaper but not standardized cardboard tools. However, it requires research to develop a prototype Kato Katz modification kit that is effective, cheap, and standardized for STHs infection early detection.

This research aims to create a laboratory-scale modified Kato-Katz kit for early detection of STHs infection by fecal egg counts (FECs) in estimating the number of eggs per gram of stool (EPG).

MATERIALS AND METHODS

This research was experimental with a diagnostic approach to create a laboratory-scale modified Kato-Katz kit for early detection of STHs infection by fecal egg counts (FECs) in estimating the number of eggs per gram of stool (EPG). An experimental study in the first year was conducted to create a resin Kato Katz template, spatulas, 60 mesh stainless sieve, 3M adhesive tape, and the malachite green in aqua-glycerol for STHs eggs in stool detection and counting them.

From June 30th to September 15^{th,} 2021, this study was conducted in Parasitology Laboratorium at Poltekkes Kemenkes Banjarmasin to create all components kits. Identification modified resin Kato Katz template quality and experience sharing from the experts' medical laboratory technician in Parasitology Laboratorium at Health Laboratory of South Kalimantan Province. The researcher measured the modified Kato-Katz resin template at Health Development Research Center Tanah Bumbu, Batu Licin, for diagnostic tests and experience sharing from the expert's researcher.

The instrument used in this study was calibrated analytical balance for stool weighing. The following two Kato-Katz templates were compared: modified Kato-Katz resin template and standard WHO template. As summarized in Table 1, the two templates are small square plastic tiles (30 mm × 40 mm) 1,5 mm thin with a central hole diameter of 6 mm. To obtain a total of 60 weighings per template type, and in line with the pioneering study published by Katz, we selected poll stool samples in Parasitology laboratories at Health Development Research Center Tanah Bumbu, Batu Licin, and prepared a total of 30 Kato-Katz thick smears from a stool sample, with each duplication of two templates under investigation. Hence, in each laboratory, we performed 120 weighings in total. Stool samples were only eligible for inclusion in the study when they were of sufficient size to prepare a total of 30 Kato-Katz thick smears. The average amount of stool generated on the slide was calculated for each template.

One Hundred and twenty Kato-Katz thick smear preparations were done; 30 per kit template resin and standard with Empty duplication slides, slides plus Kato-Katz template filled with stool and slides plus stool after careful removal of the template were weighed on an analytical balance. Place one piece of adhesive, soaked overnight in the malachite green in aqua-glycerol, over the stool sample. The composition stain is 1 ml of 3% aqueous malachite green is added to 100 ml of glycerol and 100 ml of distilled water and mixed well.

Invert the microscope slide and firmly press the sample against the strip on another microscope slide or on a smooth, hard surface to spread the stool in a circle, lifting it off. Place the slide on the bench with the cellophane upwards, keep the slide for one hour at room temperature to clear the fecal material before examination under the microscope. The smear was analyzed microscopically within 60 minutes using Olympus CX33 trinocular light microscope at 100x and 400x magnification. The presence of the STHs eggs was identified and counted all over the smear. STHs eggs were identified based on eggshells morphology and the distinctive features of STHs eggs, i.e., *Ascaris lumbricoides, Hookworm* (*Ancylostoma duodenale and Necator americanus*), and *Trichuris trichiura*. Then, multiply by the appropriate number based on average stool weighing to EPG unit or 1 gram or 1000 mg.

Data collected and edits to check completeness, continuity, and validation data. Data entered into a computer program, data analysis obtained in primary data, namely stool weighing result of Kato Katz resin template and standard template. Statical analyses used a T-test and computed the diagnostic parameter test for comparing both templates.

This study was approved by Health Research Ethics Committee Poltekkes Kemenkes Banjarmasin (HREC-PKB) No.064/KEPK/2021. After explaining the objective study, informed consent was obtained from the Head of Parasitology Department at Health Development Research Center Tanah Bumbu, Batu Licin.

RESULTS AND DISCUSSION

Table 1. Specification of the Modified Kato-Katz Resin Template and Standard WHO Template (Serenity®)

Kato Katz Templates	Modified Resin Template	Standard Template
Shape of Hole	Cylinder	Cylinder
Diameter of Hole	6 mm	6 mm
Thickness of template	1,5 mm	1,5 mm
Manufactured	Author Created	Serenity®

Table 2. Stool Weight in Modified and Standard Template of Kato-Katz

Weight (mg)	Modified Kato-Katz	Standard Kato-Katz
Mean	42.817	42.930
Standard Deviation	5.2866	31537
Minimum Value	43.300	42.200
Maximum Value	42.700	43.300
Median	42.700	42.500

Table 3. The Proportion of STHs infection stools with Modified and Standard Kato-Katz

STHs Species	Modified Kato-Katz		Standard Kato-Katz	
	N	%	N	%
Ascaris lumbricoides	12	40.00	12	40.00
Trichuris trichiura	8	26.66	8	26.66
Hookworm	3	10.00	3	10.00
Uninfected	7	23.34	7	23.34

Table 4. Mean of STHs Eggs Number in stools with Modified and Standard Kato-Katz

STHs Species	Modified Kato-Katz mean (min-max)	Standard Kato-Katz mean (min-max)	*p-value*
Ascaris lumbricoides	**12 (2-25)**	**12 (1-25)**	0.027*
Trichuris trichiura	**8 (1-21)**	**8 (2-23)**	0.038*
Hookworm	**1(1-1)**	**3 (2-3)**	0.102

*p-value < 0.05: significance correlation
*Wilcoxon Test

Table 5. Mean of STHs EPG in stools with Modified and Standard Kato-Katz

STHs Species	STHs EPG in Stool Modified Kato-Katz mean (min-max)	STHs EPG in Stool Standard Kato-Katz mean (min-max)	*p-value*
Ascaris lumbricoides	203 (47-585)	174 (24-600)	0.156
Trichuris trichiura	138 (23-491)	162 (48-552)	0.012*
Hookworm	23 (23-23)	56 (48-72)	0.102

*p-value < 0.05: significance correlation
*Wilcoxon Test

Table 1 shows the specification of the modified Kato-Katz resin template produced from resin material. A square template 3 x 4 cm in size with a cylinder hole of 6 mm in diameters on a 1.5 mm thickness. Table 2 showed the average amount of stool generated on slides was 42.817 mg (95 % confidence interval (CI): 42.0–43.4 mg) for the resin template, compared to the standard template was 42.930 mg (95 % CI: 42.2–43.3 mg. There was no difference between the resin template with the standard (p = 0,924 (>0,05).

The World Health Organization (WHO) recommends using the Kato-Katz technique to diagnose and measure worm infections with feces specimens because the technique provides standard readings of eggs per gram of feces (EPG). The

technique can be taught to microscopic laboratory experts with relative ease. This technique uses a small spatula and a slide template or stool mold that allows a standard amount of stool to be examined under a microscope, and the eggs are counted. Although this technique can be performed in the field without advanced engineering or laboratory equipment, it requires many human resources and expertise in microscopy (Turner, H.C. et al., 2017).

However, the same multiplication factor in estimating EPGs hardly influences the classification into infection intensities. More important factors for reliable EPGs, and hence intensity category results, are the careful preparation of Kato-Katz thick smears and, in particular, the accurate counting of helminth eggs on a thick smear examined under a microscope. The result in this study (Table 2) shows that the average stool weight was 42.817 mg, according to WHO Bench Aids for the diagnosis of intestinal parasites, second edition (2019) multiplication factor by the appropriate number to give the number of eggs per gram of feces, i.e., by 20 if using a 50 mg template; by 50 for a 20 mg template; and by 24 for a 41.7 mg template). We were determined that the appropriate number of multiplication factors was 23.4. Its factor calculated from 1 gram divide stool weight average in each template hole (42.817 mg/1000mg = 23.4).

The study by Leuenberger et al. in 2016 compared the average amount of feces produced by three Kato-Katz templates included in testing kits from two different providers. The average amount of stool produced on the slide is calculated for each template, grouped by standard categories of fecal consistency (i.e., flabby, soft, sausage-shaped, complex, and clumpy). The average amount of feces produced by the three specific Kato-Katz templates is about the same: 40.3-42.8 mg.

In a study by Moser et al. (2018), the sensitivity to identify any STHs infection was considerably higher for Kato-Katz compared to FECPAK^{G2} floatation. The same result can also be seen in a recent study by Allam et al. (2021), which compares Kato Katz's technique with the floatation technique and FECM with significant differences. Kato-Katz diagnosed the highest helminthic infection rate (57.8%), followed by FLOTAC FS7 (44.4%) and FECM showed the lowest helminthic infection rate (27.7%) because of more samples of stool in Kato-Katz compared with another method.

Table 3 showed no differences in the results presence of specific species of STHs infection in stool between Modified and Standard Kato-Katz. Based on both microscopic examinations, STHs proportion of positive samples infected by *Ascaris lumbricoides* is 12 (40.00%)*, Trichuris trichiura* is 8 (26.66%)*,* hookworm is 3 (10.00%), and uninfected 7 (23.34%). According to WHO and Swiss Tropical and Public Health Institute guidelines for quality control of fecal egg counts based on Kato-Katz, results are considered consistent if there is no difference in the presence/absence of a specific helminth species (Vlaminck, 2020).

Table 4 shows the Wilcoxon Test result that compares the number of STHs Eggs between modified and standard Kato-Katz. There were no differences for *Ascaris lumbricoides* eggs p-value= 0.027 and *Trichuris trichiura* eggs p-value= 0.038 ($p<0.05$), meanwhile the differences for hookworm eggs p-value=0.102 ($p>0.05$). The difference might be due to changes in the intensity level of infection as the moderate and high-level groups slightly decrease in the modified Kato-Katz kit.

Table 5 shows the Wilcoxon Test result that compares the number of EPG between the modified Kato-Katz kit and the standard. There were no differences for *Trichuris trichiura* eggs p-value= 0.012 ($p<0.05$), meanwhile the differences for *Ascaris lumbricoides eggs* p-value=0.156*,* and hookworm eggs p-value= 0.102 ($p>0.05$). Under microscopic examination, some *A.lumbricoides* eggs morphology was

not visible because the adhesive in modified Kato-Katz blurry it. According to microscopic results, identified and clearly, fewer than in standard. The *Ascaris lumbricoides'* eggshells with three layers or even its decorticated, its shape not spherical or oval, the identification process could still be performed well. The observation showed that Trichuris trichiura eggs' shape and layer structure did not change in both Kato Katz.

In this study, the mean number of hookworm eggs was only one by modified Kato-Katz, it significant difference with the standard that was 3, perhaps the hookworm eggs were detected had defects or had faded egg's layer, and the Hookworm fragile eggshells made it disappear in Kato-Katz smear within less than 60 minutes. According to WHO (2013) and Swiss Tropical and Public Health Institute guidelines for quality control of fecal egg counts based on Kato-Katz, results are considered consistent if there is a difference of egg counts exceeding less than ten eggs.

This study showed that the mean number of *Ascaris lumbricoides* eggs and EPG in stool examined by the modified Kato-Katz kit was lower than expected. The morphology of *Trichuris trichiura* eggs found during observation showed no visible defect of the eggshells; therefore, its identification process in stools was easy to do. *Trichuris trichiura* eggs have a small, tight and intact structure. Therefore, the difference in the intensity of *Trichuris trichiura* infection was not as significant as the intensity of *Ascaris lumbricoides* infection. Related to hookworm eggs, since its eggs have thin layers and fragile eggshells, examination; and consequently, this affected the incidence rate and EPG in stools. This study showed some variation in the proportion of *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm infection based on both Kato-Katz methods. *Ascaris lumbricoides* and *Trichuris trichiura* eggs in stools examined by modified Kato Katz kit were detected in fewer numbers than those examined by standard Kato-Katz (Soares et al., 2020).

Accurate diagnosis and counting of infection are essential for informing and assessing treatment interventions. The Kato–Katz technique may be less sensitive in a setting of lower intensity STH infections. Kato-Katz's thick smear technique, the most widely used laboratory method for quantitatively assessing the prevalence and intensity of infection, is often compared to other methods (Liu, C. et al., 2017).

Reduced sensitivity could be the cyclical egg-laying nature of helminths, which results in daily fluctuation in the number of eggs excreted in the feces. The daily egg production of *Ascaris lumbricoides females* may vary between 73,000 and 227,000 eggs on six consecutive days. However, *Trichuris trichiura* and hookworm, a much smaller number of helminth eggs, are excreted over days. They may escape detection in the small number of feces examined with the Kato–Katz smear. There was a high probability of false-negative with the Kato-Katz method if only one fecal sample was obtained (Barenbold et al., 2017).

According to WHO Diagnostic Technical Advisory Group for Neglected Tropical Diseases, a recent tool for STHs infection, Kato–Katz microscopy is widely used and work reasonably well for most infections Ascariasis, trichuriasis, hookworm infections except strongyloidiasis. It is standardized as the gold standard; an improved test is desired, but it is not preventing progress; fecal samples are suboptimal (easily obtained from children but not adults) and poor sensitivity for low-intensity samples (WHO, 2020).

The limitation of this research is the lack of stratified by standard categories of stool consistency (i.e., mushy, soft, sausage-shaped, complex, and clumpy). This study only compared the weighing results of clumpy stool weight between the modified Kato Katz resin template, comparing the WHO standard as the gold standard.

CONCLUSION

The study concluded no difference in EPG for *T.trichiura* eggs by FECs between modified Kato-Katz as this study product with the standard. It is recommended to improve modified Kato-Katz in further study, especially the adhesive tape, before using it as a quantitative tool for early detection of STHs infection in the field survey.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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