Jurnal Sain Veteriner, Vol. 44. No. 2. Agustus 2026, Hal. 304-309
DOI: 10.114063/jsv.110762
ISSN 0126-0421 (Print), ISSN 2407-3733 (Online)
Tersedia online di https://jurnal.ugm.ac.id/jsv

Optimization Of Velocity Variation For *Soil-Transmitted Helminth* Egg Detection Using The Formaldehyde–Detergent Sedimentation Method

Optimalisasi Kecepatan Sentrifugasi untuk Deteksi Telur Soil-Transmitted Helminths Menggunakan Metode Sedimentasi Formaldehida–Deterjen

Acivrida Mega Charisma[1*], Theresia Feby Auralya[1*], Yohanes Ardian Kapri Negara[1*], Farida Anwari[1*]

[1]D3 Medical Laboratory Technology, Faculty of Health Sciences, Anwar Medika University
Jl By Pass Krian KM 33 Sidoarjo, Jawa Tengah, Indonesia
*Email: acie.vrida@uam.ac.id

Article Submitted: August 28, 2025; revised: August 10, 2026; accepted: August 11, 2026

Abstrak

Infeksi cacing usus masih menjadi masalah kesehatan masyarakat di daerah tropis, terutama yang disebabkan oleh Soil Transmitted Helminths (STH) dengan spesies *Ascaris lumbricoides*, *Trichuris trichiura*, dan Hookworm. Metode pemeriksaan feses yang akurat sangat penting untuk mendukung diagnosis dan program pengendalian kecacingan. Tujuan: Penelitian ini bertujuan untuk mengevaluasi efektivitas variasi kecepatan sentrifugasi dengan metode sedimentasi formaldehida 15%–deterjen 5% dalam mendeteksi telur STH. Metode: Penelitian dilakukan di Laboratorium Biologi Farmasi Universitas Anwar Medika, Sidoarjo dengan desain eksperimental deskriptif. Tiga jenis sampel suspensi diperiksa dengan 92 kali pengulangan. Pemeriksaan mikroskopis menggunakan metode sedimentasi formaldehida 15%–deterjen 5% dengan variasi kecepatan sentrifugasi 2000, 3000, 4000, dan 5000 rpm selama 5 menit. Identifikasi telur cacing dilakukan berdasarkan jumlah dan morfologi khas *A. lumbricoides*, *T. trichiura*, dan hookworm. Analisis data menggunakan uji Shapiro-Wilk (p = 0,05) untuk menilai perbedaan awal antarkelompok, kemudian dilanjutkan dengan ANOVA (p = 0,05) apabila data memenuhi asumsi normalitas dan homogenitas. Hasil: Hasil penelitian menunjukkan bahwa variasi kecepatan sentrifugasi memengaruhi jumlah telur yang terdeteksi. Kecepatan 3000 rpm selama 5 menit memberikan hasil paling optimal dengan sensitivitas yang lebih tinggi dibandingkan variasi lainnya. Analisis lebih lanjut menunjukkan bahwa kecepatan 3000 rpm selama 5 menit menghasilkan jumlah telur yang lebih optimal dibandingkan variasi kecepatan lainnya (2000, 4000, dan 5000 rpm). Kesimpulan: Metode sedimentasi formaldehida 15%–deterjen 5% dengan kecepatan sentrifugasi 3000 rpm selama 5 menit meningkatkan sensitivitas pemeriksaan feses terhadap infeksi cacing usus, sehingga dapat digunakan sebagai metode alternatif yang lebih akurat untuk diagnosis laboratorium.

Kata kunci: deterjen 5%; kecepatan sentrifugasi; metode sedimentasi; pemeriksaan feses; STH; formaldehida 15%,

Abstract

Intestinal worm infections remain a public health problem in tropical areas, particularly those caused by Soil Transmitted Helminths (STH), including *Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm. Accurate stool examination methods are important to improve parasite detection and support helminth control programs. This study aimed to evaluate the effect of centrifugation speed variations using the 15% formaldehyde–5% detergent sedimentation method on the recovery of STH eggs from stool samples. This study was conducted at the Pharmaceutical Biology Laboratory of Anwar Medika University, Sidoarjo, using a descriptive experimental design. The samples used were laboratory-prepared positive stool suspensions containing STH eggs obtained from confirmed positive samples, consisting of [insert number] positive samples. A total of 92 replications were obtained by repeated examination of the prepared suspensions under four centrifugation speed treatments: 2000, 3000, 4000, and 5000 rpm for 5 minutes. Microscopic examination was performed to identify and count STH eggs based on their characteristic morphology, including *A. lumbricoides*, *T. trichiura*, and Hookworm. Data analysis included the

Shapiro–Wilk normality test (p = 0.05), homogeneity test, and one-way ANOVA (p = 0.05) to determine differences among centrifugation speed variations. The results showed that centrifugation speed variations significantly affected the number of STH eggs recovered. The 3000 rpm centrifugation speed for 5 minutes produced the highest egg recovery compared with 2000, 4000, and 5000 rpm treatments. The 15% formaldehyde–5% detergent sedimentation method with centrifugation at 3000 rpm for 5 minutes yielded significantly higher recovery of STH eggs compared with other centrifugation speed variations. Further studies involving clinical diagnostic accuracy evaluation are required to determine its performance as a diagnostic method for intestinal worm infections.

Keywords: centrifugation speed; 5% detergent; 15% formaldehyde; STH, stool examination, sedimentation method

Introduction

Worm infection is a disease that is included in the neglected disease which is ignored and does not receive enough attention from the public, this happens because worms do not show significant symptoms and only appear after a long period of time.(Isyafa et al., 2023)Soil-transmitted helminths (STH) are a group of intestinal nematodes that cause worm infections. They have a life cycle that passes through the soil until they reach the infective stage and develop in the human intestine. (Manyullei et al., 2023). Some worms are classified as Soil Transmitted Helminths (STH) which cause worm infections, including roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*), hookworms (*Necator americanus* and *Ancylostoma duodenale*), and *Strongyloides stercoralis* (Fadilla, 2023). This diagnosis can be made through laboratory examination.

The diagnosis of Soil-Transmitted Helminth (STH) infection is determined based on the discovery of eggs in fecal samples through laboratory examination. Microscopic examination consists of two categories: qualitative and quantitative. Qualitative examination (direct examination) uses several methods, namely the flotation method, the tape method, the upright slide technique, and the sedimentation method. Quantitative examination is performed using the Stoll method, qualitative flotation, and the Kato-Katz method (Permatasari et al., 2020).

Determining a laboratory diagnosis for worm infections requires appropriate methods to expedite the process and ensure accurate results. One effective technique for examining worm eggs is the sedimentation method. The sedimentation method is a solution method that utilizes only gravity and centrifugal force, so that parasite eggs will settle at the bottom. Centrifugation is a technique used to separate sediment from suspension at a speed measured in rpm (revolutions per minute). The centrifugation technique has advantages and disadvantages. The advantage of the centrifugation technique is its ability to maintain the morphology of worm eggs and produce a small suspension, thus facilitating the examination process. However, the disadvantage of the centrifugation technique is the frequency of sample contamination with other components (Onesiphorus & Kusuma, 2022).

Sedimentation methods can be classified into two categories: conventional sedimentation methods that utilize gravity and usually use 0.2% NaOH or 0.9% NaCl reagents. In this study, the Allen & Ridley formaldehyde-detergent sedimentation method was applied to examine Soil-Transmitted Helminths (STH). This second method utilizes centrifugal force during the process and uses a formaldehyde-detergent mixture.

The existence of variations in centrifugation speed and time raises questions about the importance of establishing standards for centrifugation speed and time as a reference in the sedimentation method for diagnosing Soil-Transmitted Helminths (STH) eggs. The purpose of this study was to determine the differences in variations in centrifugation speed and time on the results of the STH worm egg count examination using the sedimentation method.

Research methods

Design, Place, and Time

The type of research conducted was quantitative research using an experimental design. This type of research is quantitative research with an experimental design. The stool samples were examined to detect Soil-Transmitted Helminths (STH) eggs, namely *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm. The examination was carried out using the sedimentation method with 15% formaldehyde reagent and 5% detergent.

The procedure began with the preparation of a fecal suspension in a reagent solution, followed by centrifugation at various speeds of 2000 rpm, 3000 rpm, 4000 rpm, and 5000 rpm for 5 minutes. Each treatment was performed in 92 replicates. The sediment from the centrifugation was then observed under a light microscope, and identification was made based on the typical morphology of the worm eggs. Data analysis was performed using the Shapiro–Wilk test (normality), Levene's test (homogeneity), and one-way ANOVA to determine differences between groups. Post hoc tests were performed if significant differences were found. This research was conducted at the Pharmaceutical Biology Laboratory, Anwar Medika University, Sidoarjo, in 2025.

Population and Sample

This research has obtained ethical approval with certificate number 0488HRECC.FODM/IV/2025. The research sample was a suspension of Soil-Transmitted Helminths (STH) eggs obtained from the Surabaya Center for Health Laboratory (BBLK). The sample consisted of three types of worms, namely Ascaris lumbricoides, Trichuris trichiura, and Hookworm, with a total of 3 samples, each having a volume of 1.5 ml.

Tools and materials

The tools used in the study of the effect of centrifugation speed variation test with the formaldehyde-detergent sedimentation method are masks, handscoons, centrifuges, centrifuge tubes, test tubes, and stirring rods. The supporting tools used for observing Soil Transmitted Helminths (STH) worm eggs including *Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm worms are object glass, cover glass, dropper pipette, and microscope. The materials needed are STH worm egg suspension (*Ascaris lumbricoides*, *Trichuris trichiura*, Hookworm), aluminum foil, 15% formaldehyde solution, 5% detergent solution, and distilled water.

Work procedures

Examination of Soil Transmitted Helminths (STH) eggs using the formaldehyde–detergent sedimentation method was performed by adding 5% detergent solution (laboratory grade detergent) prepared in 15% formaldehyde solution (analytical grade formaldehyde, Merck KGaA, Darmstadt, Germany) into a centrifuge tube until the volume reached 10 mL. The egg suspension was prepared from positive STH samples, and one drop of the suspension was transferred into the tube. The samples were centrifuged at different speeds of 2000, 3000, 4000, and 5000 rpm for 5 minutes.

The supernatant was discarded, and 10 mL of formaldehyde–detergent solution (1:1 ratio) was added. The mixture was homogenized and allowed to stand for 1 hour until sediment formation occurred. The supernatant was carefully removed until approximately 0.5 mL of sediment remained. The sediment was then pipetted onto a glass slide, covered with a coverslip, and examined microscopically using a 40× objective lens. All reagents used were of laboratory analytical grade unless otherwise specified.

Results and Discussion

Effectiveness of Using 15% Formaldehyde Solution-5% Detergent

The 15% formaldehyde–5% detergent sedimentation method showed more optimal examination results compared with the 10% formaldehyde control method. Variations in centrifugation speed affected the number of detected Soil-Transmitted Helminths (STH) eggs. The optimal centrifugation speed produced a higher number of recovered eggs with clearer morphological characteristics, allowing better microscopic identification of *Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm eggs. The results indicated that the 15% formaldehyde–5% detergent sedimentation method, combined with appropriate centrifugation conditions, improved egg recovery during STH examination.

The 15% formaldehyde–5% detergent sedimentation method was performed using formaldehyde solution (37%, analytical grade) diluted to 15% concentration and detergent solution (5%, laboratory grade). All reagents used in this study were analytical or laboratory grade and prepared according to the required concentrations.

However, the use of formaldehyde in too high a concentration can cause damage to the morphology of the eggs, making microscopic identification difficult (Regina et al., 2018). Furthermore, powdered detergents have a weakness in the dissolution process, although they have been shown to increase the number of eggs detected. This finding aligns with previous research that found the formaldehyde-detergent combination to be more effective than the

formalin-ether method because it can increase the number of eggs identified and is safer for laboratory use (Azzahroh, 2023).

The Effect of Centrifugation Speed and 15% Formaldehyde-5% Detergent Concentration on STH Egg Morphology

The results of the study showed that variations in centrifugation speed with a 15% formaldehyde solution and 5% detergent affected the morphological quality of Soil-Transmitted Helminths (STH) eggs. The pH of the weakly acidic formalin and the neutral-alkaline pH (7.5–10) of the detergent played an important role in maintaining the stability of albuminoid, which is amphoteric and therefore can react at both acidic and alkaline pH (Darmadi & Dikna, 2022).

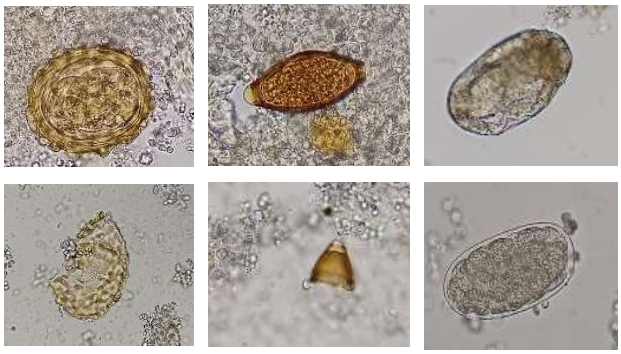
In *Ascaris lumbricoides*, a speed of 3000 rpm for 5 minutes produced the clearest morphology, with the albuminoid layer and larvae clearly visible. This finding is consistent with a study by Manser et al. (2016), who reported that a formalin-detergent combination at 3000 rpm improved microscopic visual clarity and reduced fecal debris. For *Trichuris trichiura*, variations in speed did not cause significant changes, although at 3000–4000 rpm, the morphology of the albuminoid, mucoid plug, and germ cells was clearer than at low or high speeds. This is in line with Tadege et al. (2022), who showed that the use of detergent can increase the recovery of *T. trichiura* eggs without damaging morphology. In hookworms, the 3000 rpm for 5 minutes condition provided the most optimal morphology, with a clear albuminoid layer and morula structure, in line with the study by Manser et al. (2016). Overall, a speed of 3000 rpm for 5 minutes proved to be the optimum condition for maintaining the clarity and sharpness of the morphology of *A. lumbricoides*, *T. trichiura*, and Hookworm eggs. Higher speeds (>3500 rpm) tended to produce slides with more debris, thus reducing the quality of microscopic observations.

Effect of Centrifugation Speed on the Number of Soil-Transmitted Helminths STH

The results showed that the highest number of Soil-Transmitted Helminths (STH) eggs was found in the 3000 rpm for 5 minutes centrifugation treatment for *Ascaris lumbricoides* and *Trichuris trichiura*. Meanwhile, the highest number of eggs for hookworms was found in the 2000 rpm for 5 minutes treatment. This finding is in line with the research of Onesiforus & Kusuma (2022), who observed egg morphology more optimally at a speed of 2000 rpm for 10 minutes or 3000 rpm for 5 minutes. Conversely, at high speeds (4000–5000 rpm), the number of detected eggs decreased. Ichsan et al. (2017) reported that no worm eggs were found at 4000 rpm for 5 minutes, which is likely due to the increased temperature during centrifugation, which causes egg lysis (Sebayang et al., 2020). This is also in line with the theory of uniform circular motion, where higher speeds increase the centrifugal force, potentially damaging the egg structure. Manser et al. (2016) emphasized that low speeds, with sufficient time, are more effective in maintaining parasite morphology, while high speeds can cause damage or loss of eggs.

Overall, a speed of 3000 rpm for 5 minutes was the optimum condition for detecting *A. lumbricoides* and *T. trichiura* eggs, while 2000 rpm for 5 minutes was more suitable for hookworm, as both were able to maintain egg morphology and increase the sensitivity of the assay.

The morphological appearance can be seen in Figure 1.



(a) (b) (c)

Figure 1. Morphology of Soil-Transmitted Helminths (STH) Eggs (a) Ascaris lumbricoides eggs at 3000 rpm and 5000 rpm for 56 minutes (b) Trichuris trichiura eggs at 3000 rpm and 5000 rpm for 56 minutes. (c) Hookworm eggs at 3000 rpm and 5000 rpm for 56 minutes. Magnification 1000x.

Differences in the results of the examination of the number of STH worm eggs based on centrifugation speed

The normality test was conducted using Shapiro-Wilk because the sample size was ≤ 50. The test results showed that a significance value <0.05 means the data is not normally distributed, while a significance value >0.05 indicates the data is normally distributed. The test results can be seen in Table 1.

The results of the Shapiro–Wilk normality test showed that most of the data (75%) were normally distributed, while the homogeneity of variance test

Table 1. Microscopic Observation Results of the Number of STH Eggs

Speed Variation		*Sig*	information
2000 rpm 5 minutes (T1)	*Ascaris lumbricoides*	0.006	Normal
	Trichuris trichiura	0.091	
	Hookworm	0.101	
3000 rpm 5 minutes (T2)	*Ascaris lumbricoides*	0.875	
	Trichuris trichiura	0.195	
	Hookworm	0.212	
4000 rpm 5 minutes (T3)	*Ascaris lumbricoides*	0.178	
	Trichuris trichiura	0.212	
	Hookworm	0.415	
5000 rpm 5 minutes (T4)	*Ascaris lumbricoides*	0.004	
	Trichuris trichiura	0.212	
	Hookworm	0.001	

(Levene's test) showed homogeneous data ($p > 0.05$), so that the ANOVA analysis was valid. The ANOVA test showed significant differences between treatments ($p < 0.05$), except for treatment T3 (4000 rpm) which was not significantly different ($p > 0.05$). The ANOVA statistical test can be seen in Table 2.

The results of the Shapiro-Wilk normality test showed that most of the data (75%) were normally distributed, and the homogeneity of variance test (Levene's test) showed homogeneous data ($p > 0.05$), so that ANOVA analysis could be performed. The ANOVA results showed significant differences in treatments T1, T2, and T4 ($p < 0.05$), while T3 was not significantly different ($p > 0.05$). A further Bonferroni test showed that the treatment with a speed of 3000 rpm (T2) provided the most significant difference between worm types ($p = 0.001$), covering all pairs of groups. This confirms that the speed of 3000 rpm is the most effective condition in influencing the number and morphology of worms, while T3 (4000 rpm) proved to be less effective ($p = 0.064$).

Conclusion

The 15% formaldehyde–5% detergent sedimentation method with different centrifugation speed variations showed differences in the recovery and morphological quality of Soil-Transmitted Helminths (STH) eggs. Among the tested centrifugation speeds, 3000 rpm for 5 minutes produced the highest number of recovered STH eggs with clearer morphological characteristics compared with 2000, 4000, and 5000 rpm. These findings indicate that centrifugation speed influences the recovery and microscopic observation quality of *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm eggs using the 15% formaldehyde–5% detergent sedimentation method.

Acknowledgments

We would like to thank the D3 Medical Laboratory Technology who has helped and supported this research.

References

Azzahroh, SN (2023). Differences in the Number of Worm Eggs in the Sedimentation Method Using Formol-Ether Solution and Formol-Tween. Repository.poltekkesjogja.https://poltekkesjogja.ac.id

Darmadi, & Dikna, J. (2022). Morphology of Ascaris lumbricoides Eggs Using Hematoxylin Eosin Staining. Journal of Medical Laboratory Technology (BJMLT), 5(1), 335–340.

Table 2. One-way ANOVA on the Number of Soil-Transmitted Helminths (STH) Eggs

Speed Variation	Mean square results	*Sig*	Information
2000 rpm 5 minutes (T1)	7,389 0.965	0.005	Significant ($p < 0.05$)
3000 rpm 5 minutes (T2)	15,167 1,311	0.001	Significant ($p < 0.05$)
4000 rpm 5 minutes (T3)	9,500 2,867	0.064	Not significant ($p > 0.05$)
5000 rpm 5 minutes (T4)	5,722 0.378	0,000	Significant ($p < 0.05$)

Fadilla, Z. (2023). Identification of Soil-Transmitted Helminths (STH) Eggs in Raw Vegetables in Traditional Markets. Jurnal Medical Laboratory, 2(1), 30–40.

Isyafa, FR, Mahtuti, EY, & Faisal. (2023). Examination of Soil Transmitted Helminths (STH) in the Feces of Waste Collectors in Tawangsari Village, Malang Regency. Journal of Educational Innovation and Public Health, 1(4), 152–164.

Manser, MM, Saez, ACS, & Chiodini, P.L. (2016a). Faecal Parasitology: Concentration Methodology Needs to be Better Standardized. PLoS Neglected Tropical Diseases, 10(4). https://doi.org/10.1371/journal.pntd.0004579

Manyullei, S., Girikallo, GG, Bakri, M., & Saputri, VS (2023). Worm Infection Counseling at Benteng Sanrobone Elementary School, Takalar Regency. Altifani Journal of Research and Community Service, 3(1), 13–18.

Onesiforus, BY, & Kusuma, A. (2022). The Effect of Variations in Centrifugation Duration and Speed on the Profile of Soil-Transmitted Helminth Eggs in the Sedimentation Method. Journal of Science and Health (JUSIKA), 6(2), 85-92.

Permatasari, DA, Rochiman, K., Restiadi, TI, Mumpuni, S., Suprihati, E., & Effendi, MH (2020). Prevalence and Worm Infection Degree of Gastrointestinal in Duck (Anas javanica) in Two Different Geographical Territories. Journal of Parasite Science, 4, 21–24.

Regina, MP, Halleyantoro, R., Bakrie, S., & Bakri, S. (2018). Comparison of Stool Examination Between the Ordinary Sedimentation Method and the Formol-Ether Sedimentation Method in Detecting Soil-Transmitted Helminths. Diponegoro Medical Journal, 7(2), 527–537.

Sebayang, R., Idawati, Y., & Sinaga, H. (2020). Analysis of Lactate Dehydrogenase in Blood Serum Using Centrifugation. Silampari Nursing Journal, 4(1), 274–280.