

# Tropical Medicine Journal

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- Risk Factors Analysis of Typhoid Fever Occurrence of Inpatient in Kebumen Public Hospital in 2013
- Knowledge, Attitude and Practice on Dengue Fever Transmission Among Urban and Periurban Residents of Dhaka City, Bangladesh
- Geographic Information System (GIS) for Dengue Research in Indonesia: A Review
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## Comparing the Sensitivity and Specificity of Zinc Sulphate Flotation Method to Formol Ether Sedimentation Method in Identifying Intestinal Protozoa's Cysts

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Dini Alyani<sup>1</sup>, Elsa Herdiana Murhandarwati<sup>2</sup>, Sri Sumarni<sup>2</sup>, Ernaningsih<sup>2</sup>.

<sup>1</sup>Undergraduate Student, Faculty of Medicine, Universitas Gadjah Mada; <sup>2</sup>Department of Parasitology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Corresponding author: [dinialyani@live.com](mailto:dinialyani@live.com)

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### ABSTRACT

**Introduction:** The two main methods to detect the presence of intestinal protozoa's cysts are zinc-sulphate flotation (Faust's) and formol-ether sedimentation (Ritchie's) methods. Some researchers have recommended using both flotation and sedimentation in detecting the intestinal parasites. However, this approach is unpractical for most laboratories. To date there are no studies that conclude which method, either Faust's method or Ritchie's method, is more effective in term of sensitivity and specificity of detecting the intestinal protozoa cyst. This study was done to conclude which method has higher sensitivity and specificity

**Objectives:** To compare the sensitivity and specificity of Faust's to Ritchie's methods in identifying intestinal protozoa.

**Methods:** Thirty anonymous fecal samples were obtained from Parasitology Laboratory Faculty of Medicine Gadjah Mada University. Each sample was tested using both Faust's and Ritchie's methods and then microscopically examined to find the intestinal protozoa's cysts. Numbers of samples with cyst-positive were recorded to determine which method has higher ability to detect the intestinal protozoa's cysts. Data was analyzed by calculating sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

**Results:** From all the five identified cysts (*Entamoeba histolytica*, *Giardia lamblia*, *Entamoeba coli*, *Iodamoeba butschlii*, *Blastocystis hominis*), Ritchie's method was able to detect more cyst than Faust's method. The sensitivity and specificity of Ritchie's method compared to Faust's were 100% and 93.33% respectively. The positive predictive value (PPV) and negative predictive value (NPV) of Ritchie's compared to Faust's method were 93.75% and 100% respectively. Ritchie's method is proven to have higher sensitivity and specificity than Faust's method. This method has high sensitivity probably because of the cysts are concentrated and so more cysts could be collected.

**Conclusion:** This study concludes that the Ritchie's method has higher sensitivity and specificity compared to Faust's method in detecting five common intestinal protozoa's cysts.

**Keywords:** formol-ether sedimentation method, zinc-sulphate flotation method, intestinal protozoa, sensitivity, specificity.

## INTISARI

**Pendahuluan:** Terdapat dua metode utama untuk mendeteksi kista protozoa intestinal yaitu metode pengapungan zink sulfat (*zinc sulphate floatation*/Metode Faust) dan metode sedimentasi formol eter (*formol ether sedimentation*/Metode Ritchie). Para peneliti merekomendasikan kedua metode tersebut untuk mendeteksi parasit intestinal, tetapi pendekatan ini kurang praktis untuk sebagian besar laboratorium. Sampai saat ini belum ada penelitian yang menyimpulkan metode yang lebih efektif untuk mendeteksi kista protozoa intestinal dalam hal sensitivitas maupun spesifitas. Penelitian ini dilakukan untuk menyimpulkan metode yang mempunyai sensitivitas dan spesifitas yang lebih tinggi.

**Tujuan:** Untuk membandingkan sensitivitas dan spesifitas Metode Faust dan Ritchie dalam mengidentifikasi kista protozoa intestinal.

**Metode:** Tigapuluh sampel feses anonim diperoleh dari Laboratorium Parasitologi Fakultas Kedokteran Universitas Gadjah Mada. Setiap sampel diuji dengan Metode Ritchie dan Faust dan kemudian diperiksa secara mikroskopis untuk mendeteksi adanya kista protozoa intestinal. Jumlah sampel dengan positif kista dicatat untuk menentukan metode yang lebih baik. Data dianalisis dengan menghitung sensitivitas, spesifitas, nilai ramal positif (*positive predictive value*/PPV) dan nilai ramal negatif (*negative predictive value*/NPV).

**Hasil:** Dengan menganalisis lima kista protozoa yang dapat diidentifikasi (*Entamoeba histolytica*, *Giardia lamblia*, *Entamoeba coli*, *Iodamoeba butschlii*, *Blastocystis hominis*), Metode Ritchie dapat mendeteksi lebih banyak kista dibandingkan dengan Metode Faust. Sensitivitas dan spesifitas Metode Ritchie dibanding Metode Faust adalah 100% and 93,33% . Nilai ramal positif dan nilai ramal negatif Metode Ritchie dibanding dengan Metode Faust adalah 93,75% and 100%. Metode Ritchie terbukti memiliki sensitivitas dan spesifitas yang lebih tinggi dibandingkan dengan Metode Faust. Pada penelitian ini Metode Ritchie lebih sensitif kemungkinan karena sampel yang mengandung kista dipisahkan terlebih dahulu sehingga lebih banyak kista yang dapat dikumpulkan.

**Simpulan:** Metode Ritchie memiliki sensitivitas dan spesifitas yang lebih tinggi dibandingkan dengan Metode Faust dalam mendeteksi lima protozoa intestinal umum.

**Kata Kunci:** metode pengapungan zink-sulfat, metode sedimentasi formol-eter, protozoa intestinal, sensitivitas, spesifitas

## INTRODUCTION

Indonesia is located in tropical area of the world where intestinal parasitic infection is common to be encountered especially in rural areas<sup>1,2</sup>. Protozoa infection is one of the most commonly found infections<sup>3</sup>. The commonly found intestinal protozoa are *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli*, *Entamoeba coli*, and *Iodamoeba butschlii*.

A simple microscopic examination of stool should be carried out for the diagnosis of intestinal protozoa infection. But when a negative result is obtained from the examination of stained preparations of a direct smear, the utilization of a concentration method should be performed and often gives a positive result<sup>1</sup>. There are two common methods that routinely performed in laboratory which are flotation and sedimentation. Some researchers



recommend using both flotation method and sedimentation procedures since neither technique alone can identify all parasites in the fecal samples. However this approach is unpractical for most laboratories<sup>4</sup>.

The accuracy of a diagnostic tool is represented by the high sensitivity and specificity values. The more accurate it is, the higher sensitivity and specificity it has. Sensitivity is how many percent a diagnostic tool correctly diagnosed its positive results possessing the disease. On the other hand, specificity is percentage of negative results that correctly diagnosed as true negative or truly do not possess the disease.

For the reasons that has been elaborated in the previous paragraphs, determining which method that more effective in terms of sensitivity and specificity, is essential to be done and might offer positive contribution to the laboratory practice in the future.

## MATERIALS AND METHODS

Thirty anonymous formalin-preserved fecal samples were obtained from Parasitology Laboratory in Faculty of Medicine at Gadjah Mada University. Each sample was tested using both formol-ether sedimentation (Ritchie's method) and zinc-sulphate (Faust's method) and then microscopically examined to find the intestinal protozoa cysts. Numbers of samples with cyst-positive were recorded to determine which method has higher ability to detect the intestinal protozoa cyst.

In this study, sensitivity is defined as number of samples that are positive in both methods (true positive results) divided by number of samples that are positive in Faust method. A Positive sample is the sample with the presence of any species of intestinal protozoa cyst (*Entamoeba histolytica*, *Giardia lamblia*, *Iodamoeba butschlii*, *Entamoeba coli*, *Blastocystis hominis* and *Balantidium coli*). A Negative sample is sample that contains no cyst. Specificity is defined as number of negative samples that are negative (true negative results) in both method divided by number of samples that are negative in Faust method. Negative predictive value (NPV) definition is the probability of true negative result correctly detected by the tested-method. Positive predictive value (PPV) is the probability of true positive result correctly detected by the tested-method. In practical language, NPV is defined as true negative results divided by negative results detected with Ritchie method. On the other hand, PPV is true positive results divided by positive results detected with Ritchie method.

## RESULTS AND DISCUSSIONS

The results showed that there were five intestinal protozoa's cysts found in the study namely *Entamoeba histolytica*, *Giardia lamblia*, *Iodamoeba butschlii*, *Entamoeba coli*, and *Blastocystis hominis*. *Balantidium coli* was not found anywhere in the 30 samples yet *Blastocystis hominis* is unexpectedly detected (Table 1).

Table 1. Number and percentage of identified intestinal protozoa's cysts using Ritchie's and Faust's methods

Intestinal Protozoa	Ritchie		(n = 30)		Faust	
	+	-	+	-	+	-
Eh	10	20	5	25	5	25
Gl	10	20	7	23	7	23
Bc	0	30	0	30	0	30
Ec	5	25	2	28	2	28
lb	4	26	0	30	0	30
Bh	8	22	6	24	6	24

Notes: Eh = *Entamoeba histolytica*

Gl = *Giardia lamblia*

Bc = *Balantidium coli*

Ec = *Entamoeba coli*

lb = *Iodameba butschlii*

Bh = *Blastocystis hominis*

+ = Number of protozoa present in the 30 samples

The microscopic appearance of intestinal protozoa's cysts detected in this study was documented to analyse the reliability of identification. Several findings were

documented and the viewpoint was chosen very carefully for the clear depicting. Unfortunately, the representative pictures are not as clear as it was in microscope view.



Figure 1. Iodine-stained microscopic view of *Entamoeba histolytica*'s cyst (40X Objective lens magnification)



Figure 2. Iodine-stained microscopic view of *Giardia lamblia*'s cyst (40X objective lens magnification)

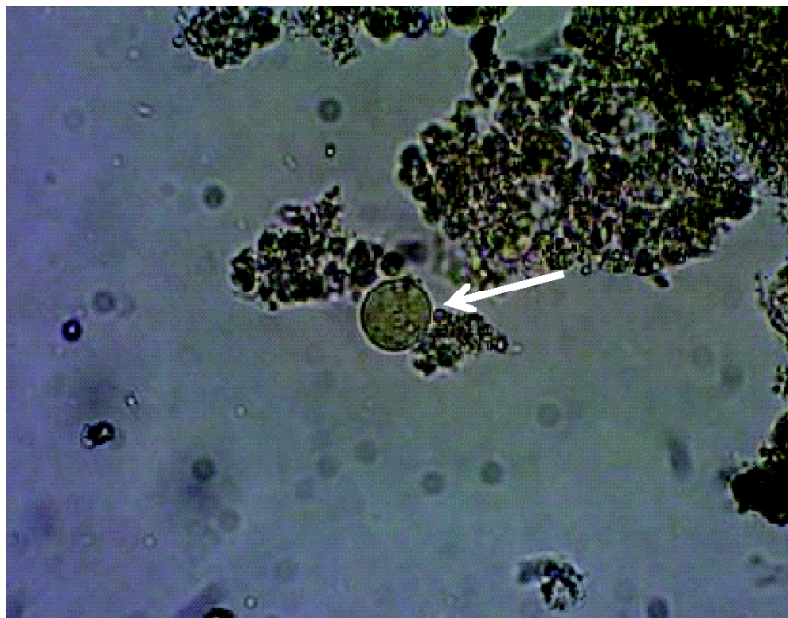


Figure 3. Iodine-stained microscopic view of *Entamoeba coli*'s cyst (40X objective lens magnification)



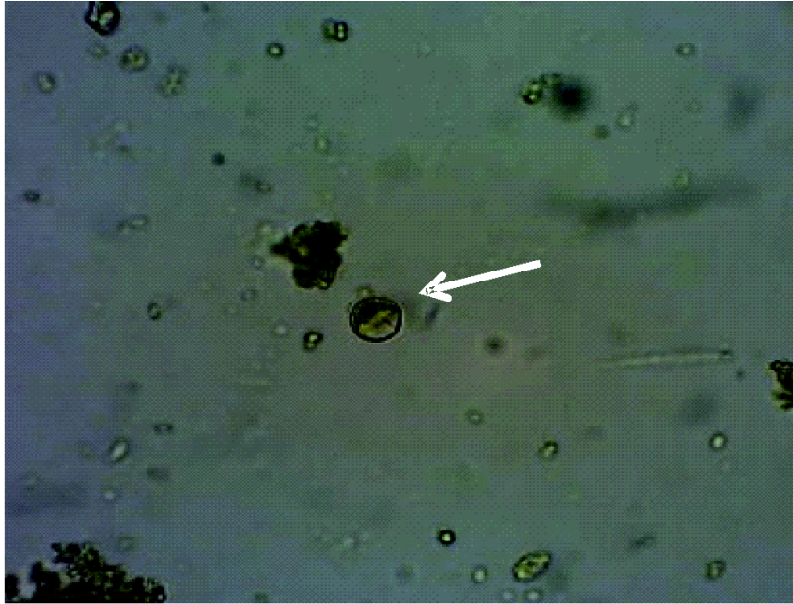


Figure 4. Iodine-stained microscopic view of *Iodamoeba butschlii*'s cyst (40X objective lens magnification)

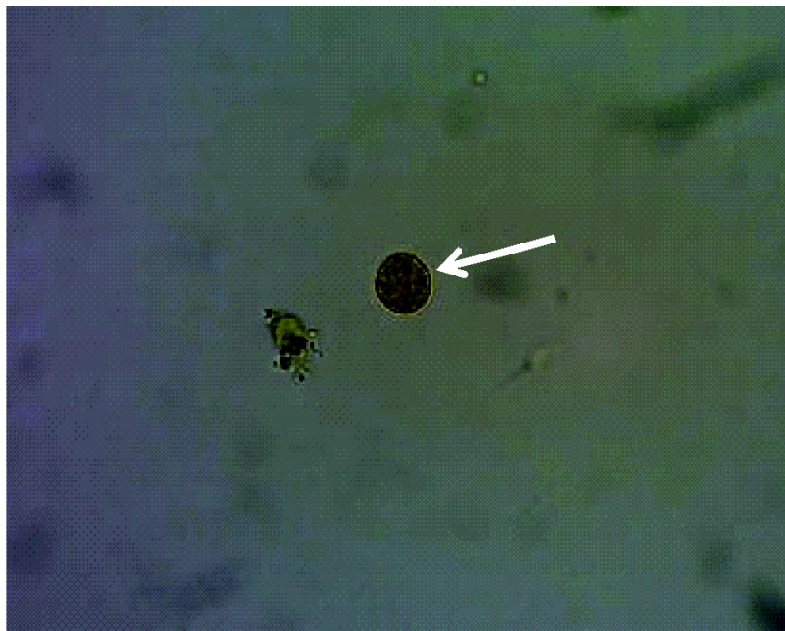


Figure 5. Iodine-stained microscopic view of *Blastocystis hominis*'s cyst (40X objective lens magnification)

Table 2. Cross table for sensitivity and specificity of Ritchie's compare to Faust's methods

		Ritchie (tested-method)		
		Positive	Negative	Total
Faust	Positive	15 (TP)	0 (FN)	15
	Negative	1 (FP)	14 (TN)	15
Total		16	14	30

Notes: TP = true positive  
 FP = false positive  
 FN = false negative  
 TN = true negative

In this study, the statistical indicators analyzed were sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The sensitivity and specificity (Table 2 and Table 3) of Ritchie's method compared to

Faust's were 100% and 93.33% respectively. The PPV and NPV of Ritchie's method compared to Faust method were 93.75% and 100% respectively.

Table 3. Statistical indicators of tested-method (Ritchie's method)

No	Indicator	Formula	Calculation	Results
1	Sensitivity	$TP/(TP + FN)$	15/15	100%
2	Specificity	$TN/(TN + FP)$	14/15	93.33%
3	NPV	$TN/(TN + FN)$	14/14	100%
4	PPV	$TP/(TP + FP)$	15/16	93.75

Ritchie's method is proven to have high sensitivity and specificity. This method has high sensitivity probably because the cysts are concentrated and more cysts could be collected. Our findings agree with the study done by Perry et al. (1990) stated that the fecal material distribution and cleaning are important factors influencing parasite detection<sup>5</sup>. Nevertheless, there is a study that argued the disadvantage of sedimentation techniques is the production of a sample containing a large amount of debris, which makes parasitic elements difficult to be identified <sup>4</sup>.

The sensitivity and specificity of Ritchie's compare to Faust's is 100% and 93.33% respectively which are considered has higher ability to detect all five intestinal protozoa's cysts. It mean that this method is very good in detecting the common intestinal protozoa's cyst compared to Faust's method and has correctly identified the cysts. The NPV and PPV value of Ritchie's method is also high so this method is able to correctly assess positive results as such and rarely misclassifies positive as negative results.

## CONCLUSION

This study concludes that the Ritchie's method has higher sensitivity and specificity compared to Faust's method in detecting five common intestinal protozoa's cysts.

## RECOMMENDATION

This study has only 30 samples of specimens so that any future study is recommended to have more significant number of samples. The tested-methods might also be compared with the direct microscopic exam so that the comparisons are equitable.

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..... according to Sardjito<sup>2</sup> .....

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## Sample References

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Dr. Dra. Erna Kristin, Apt., M.Si.

dr. Hanggoro Tri Rinonce, Ph.D

dr. M. Lutfan Lazuardi, M.Kes., Ph.D

dr. Titik Nuryastuti, Ph.D, Sp.MK

dr. Tri Wibawa, Ph.D

Dra. Raden Ajeng Yayi Suryo Prabandari, M.Si., Ph.D