Tropical Biomedicine 27(2): 177–184 (2010)

Evaluation and modification of the formalin–ether sedimentation technique

Uga, S.*, Tanaka, K. and Iwamoto, N.
Department of Parasitology, Faculty of Health Sciences, Kobe University Graduate School of Health Sciences, 7-10-2, Tomogaoka, Suma-ku, Kobe 654-0142, Japan
* Corresponding author email: ugas@kobe-u.ac.jp
Received 9 December 2009; received in revised form 24 February 2010; accepted 1 March 2010

Abstract. Formalin–ether sedimentation (MGL) is a well-known technique for the examination of faeces for parasites, but some recent reports have indicated that its efficiency is not as high as originally thought. We reevaluated the recovery efficiency of the original MGL (O-MGL) technique to modify it. We subsequently adopted the following modified MGL technique (M-MGL): filtration by three layers of gauze and washing, adjustment to pH 3, retreatment of plug, and use of 1.5 g of faeces. We also compared five faecal examination techniques (including the O-MGL and the M-MGL) for three parameters: recovery efficiency, sensitivity, and mean number of eggs detected. The highest sensitivity was obtained by the M-MGL (95%), followed by the commercially available kit (Kit; 90%), O-MGL (76%), Kato–Katz (KK; 57%), and direct smear (DS; 50%). The mean numbers of *Ascaris lumbricoides* eggs recovered by the techniques were in order M-MGL (148 eggs), Kit (97), O-MGL (41), KK (11), and DS (6). This M-MGL technique has the advantage not only of the above-mentioned three parameters, but also the ease of microscopic observation and the concentration index. The parameters of the O-MGL technique were not necessarily sufficient compared with the other techniques. It seems that the improved M-MGL technique in the present study is applicable for field surveys, particularly when the survey is done in areas of low parasite endemicity.

INTRODUCTION

It is estimated that approximately one in every four people in the world is infected with soil-transmitted nematodes such as *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm; 300 million are infected with intestinal protozoa; and 200 million are infected with schistosomes (Manson-Bahr & Apted, 1982; King *et al.*, 2006). The diseases caused by these parasites have an impact particularly on the health of children and pregnant women in "developing countries" (Chan, 1997; Montresor *et al.*, 1998; Kamimura *et al.*, 2000).

Various techniques have been used for examination of these parasitic diseases. Recently, new sensitive and time-consuming techniques based on immunology and/or molecular biology have been developed for the diagnosis of parasitic diseases. The results obtained with these techniques are not reliable because of their low specificity (Yu *et al.*, 2007; Lin *et al.*, 2008), and these techniques are expensive. With respect to soil-transmitted nematodes, the classical faecal examination is considered to be one of the most reliable techniques. More than 10 faecal examination techniques are known, including direct smear (DS) and/or concentration (Tada *et al.*, 1987). In particular, the formalin–ether sedimentation technique (406th Medical General Laboratory (MGL)), first reported by Ritchie (1948), is commonly used for detecting helminth eggs, larvae, and protozoan (oo)cysts (Ash & Orihel, 1987). According to Ritchie (1948), the recovery efficiency of the MGL technique for helminth eggs and protozoan (oo)cysts

is superior to that of the direct smear and general centrifugal sedimentation techniques. Modification of the MGL technique has been reported by Young *et al.* (1979), Parija *et al.* (2003) and Methanitikorn *et al.* (2003). This modification has been used until the present day as the one of the most useful concentration techniques for faecal examination (Tada *et al.*, 1987).

However, some reports have mentioned that the recovery efficiency of this technique is not as high as had been thought. For example, Utzinger *et al.* (2007) reported that the sensitivity of the Kato–Katz (KK) technique for hookworm eggs is higher than that of the MGL technique, and similar results have been reported with *Clonorchis sinensis* eggs (Hong *et al.*, 2003) and *T. trichiura* eggs (Goodman *et al.*, 2007). It was reported that only 3% of the total number of eggs were detected in one test of the original MGL (O-MGL) technique. If the recovery efficiency of the faecal examinations is low, an increased number of false-negative results and underestimation of parasitic diseases may result.

No study has been conducted to reevaluate quantitatively the recovery efficiency of the O-MGL technique. We therefore reevaluated the O-MGL technique using faecal samples under controlled conditions, mixed with *A. lumbricoides* fertilized eggs. We also compared the recovery efficiency, sensitivity, and mean number of eggs detected from five faecal techniques using faecal samples obtained from an endemic area.

MATERIALS AND METHODS

Faecal samples

The faeces used for condition setting of the MGL technique were collected from five Japanese male subjects who had previously been confirmed to be parasite-negative. These faeces were mixed vigorously and *A. lumbricoides* fertilized eggs were added to give an egg concentration of 730 per gram. For the comparative study, 50 faecal samples collected from an endemic area for intestinal parasites in Vietnam were used.

O-MGL technique

The technique reported by Ritchie (1948) and Yoshida (1985) was regarded as the O-MGL technique. The procedure was as follows: (1) 0.5 g of faeces was suspended with normal (0.9%) saline solution, filtered through one layer of gauze, and centrifuged at 700 × *g* for 2 min; (2) the sediment was suspended with 7 mL of 10% formalin; (3) after 30 min, 3 mL of ether was added, and shaken for 30 s; and (4) the tube was again centrifuged and the plug recovered.

Modification of the O-MGL technique

For evaluation of the recovery efficiencies of the modified MGL (M-MGL) technique, we expressed them by using plug weight (milligram) and concentration index (CI; number of eggs per 1 mg of plug). We also compared the CI and total processing time of O-MGL and M-MGL.

Filtration and washing of gauze: Type-I gauze was used for filtration of faecal suspensions. The use of the gauze was in compliance with the Japanese Pharmacopoeia. One-to-five layers of gauze were used for the filtration. Washing was carried out (if necessary) by pouring 5 mL of 10% formalin onto the gauze.

Adjustment of pH of the faecal suspension: The pH of the faecal suspension was adjusted to 3, 4, 7, and 10 with 0.1 M hydrochloric acid, calcium carbonate, and 1 M sodium hydroxide.

Post-treatment of the plug: The plug obtained from the O-MGL was regarded as a faecal sample and further treatments were undertaken. The post-treatment included addition of 7 mL of 0.1% gelatin solution or sucrose solution (specific gravity = 1.04) to the plug, and the weight of the remaining plug was measured after centrifugation at 700 × *g* for 2 min. In another experiment, the plug was filtered with 3–5 layers of gauze, and rewashing by 5 mL of formalin. The plug weight was then measured.

Comparative study

We compared recovery efficiency (of the samples examined, percent of positive samples obtained by respective techniques), sensitivity (of the positive samples examined, percent of positive samples obtained by respective techniques), and mean number of eggs detected from five faecal examination techniques using 50 faecal samples collected in an endemic area (in the case of the Kit and DS, 23 faecal samples were used). These techniques were the O-MGL, M-MGL, Kit (Fecal Parasite Concentrator; Evergreen, Los Angeles, CA, USA), KK, and DS. The plug obtained by the O-MGL and M-MGL techniques was suspended in 300 µL of formalin. Twenty microliters of the suspension was used for microscopic examination. Among the samples examined, egg-positive sample was judged to be positive regardless of species of parasite and/or number of egg. These positive samples were used for calculation of recovery efficiency and sensitivity of respective techniques. All egg numbers in positive samples were counted.

Statistical analysis

Statistical differences were analyzed using ANOVA in conjunction with the Dunnett test for *post-hoc* comparison. In some experiments, the chi-squared test was used for statistical analyses. $P < 0.05$ was considered significant.

RESULTS

Figure 1 shows the effects of filtration and washing on egg recovery. The plug weight and CI obtained using the O-MGL technique were 69 mg and 2.3, respectively. The plug weight was reduced to 46 mg when three layers of gauze were used, but CI remained at 2.3. The CI increased to 3.7 when the washing was done after the filtration, and this was significantly higher than that of the O-MGL technique (*$P < 0.01$; **$P < 0.05$) (Figure 1). This result clearly showed that the combination of three layers of gauze and washing improved egg recovery. Adjusting the pH of the faecal suspension was effective in reducing plug weight. When the pH was changed from 4 to 3, plug weight was significantly decreased from 69 mg to 47 mg ($P < 0.05$).

The effect of post-treatment is shown in Figure 2. Although the plug weight obtained was reduced by treatments of gelatin and sucrose, the CI was not improved (3.1 and 2.3, respectively). Post-treatment after filtration through 3–5 layers of gauze and washing reduced the plug weight and subsequently increased the CI (Figure 2). Filtration with three layers of gauze and washing with 5 mL of formalin (CI obtained by this post-treatment was 4.2) was adopted as post-treatment for the M-MGL technique.

For the M-MGL technique, we increased the faecal weight and evaluated the effect on plug weight and CI (Figure 3). When 1.5 g of faeces (which was three-times more than that used for the O-MGL technique) was used, the CI was significantly higher than that of the O-MGL techniques ($P < 0.05$).

We compared the two techniques of O-MGL and M-MGL. The latter improved not only the plug weight and the CI, but also the microscopic observation of the plug. The observation of eggs in the plug from the O-MGL technique was sometimes difficult because of the larger amount of debris contained therein, but this was not the case for the plug from the M-MGL technique. The CI and total processing time of the O-MGL and M-MGL techniques were 2.3, 45 min and 4.2, 49 min, respectively.

The recovery efficiency, sensitivity and mean number of egg detected by the different techniques were compared (Table 1). The recovery efficiency of O-MGL (32%) was higher than that for the KK (24%) and the DS (22%), but was lower than that for the Kit (39%) and the M-MGL (40%). The M-MGL technique showed the best results when they were compared with use of the parameter of recovery efficiency and sensitivity. However, these differences (Table 1) among the techniques were not statistically significant ($P > 0.05$). The prevalence of 50 samples used for the comparative study was 42% because 21

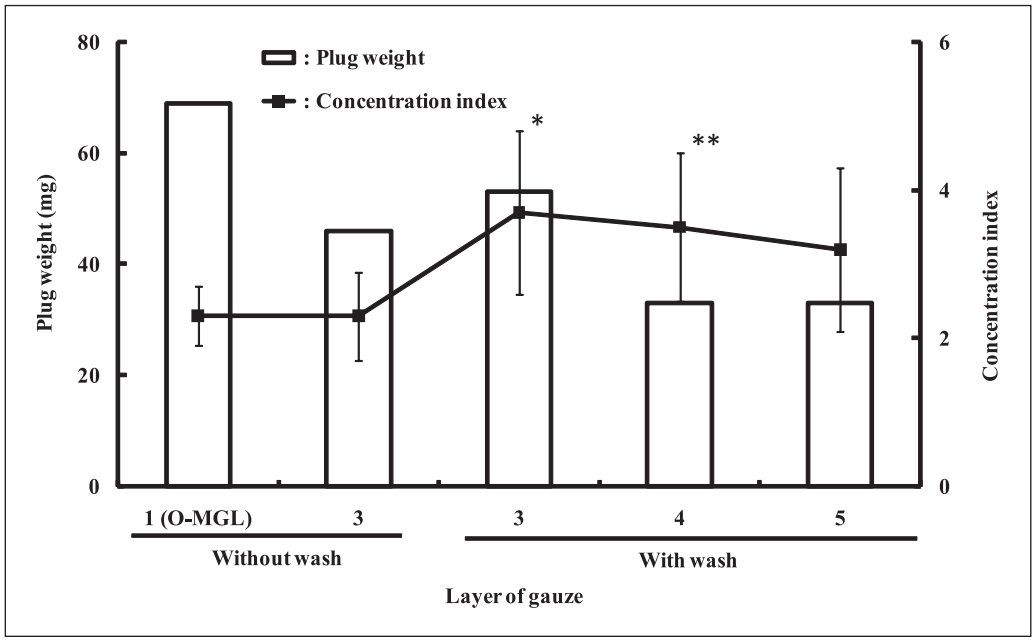

Figure 1. Effect of filtration and washing of gauze on egg recovery (*$P < 0.01$; **$P < 0.05$)

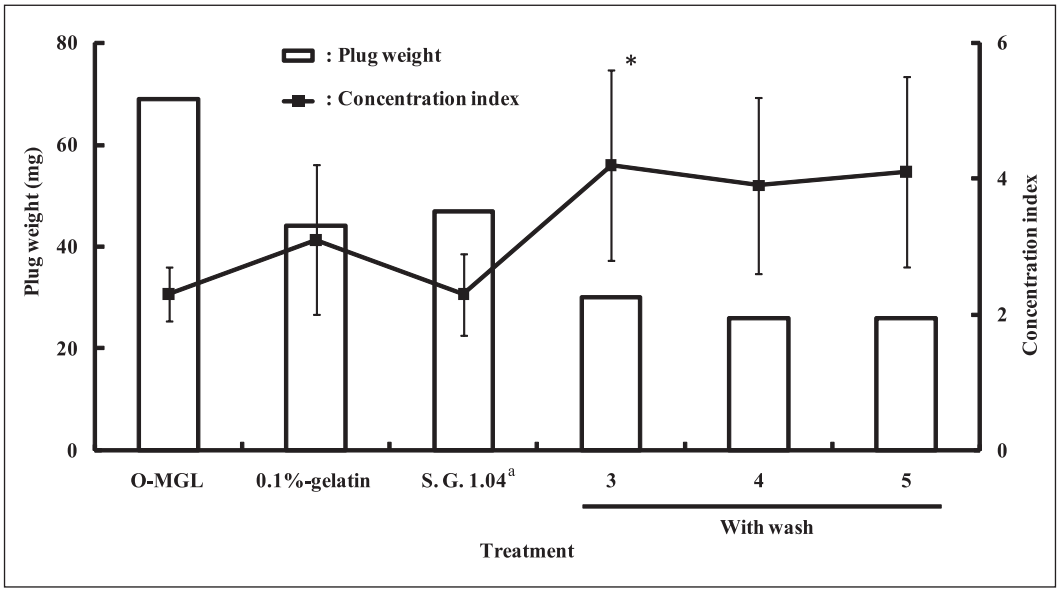

Figure 2. Effect of post-treatment on egg recovery
*Statistically significant compared with the O-MGL technique ($P < 0.05$).
[a]10% formalin adjusted the specific gravity to 1.04 by sucrose solution (S.G. 1.04).

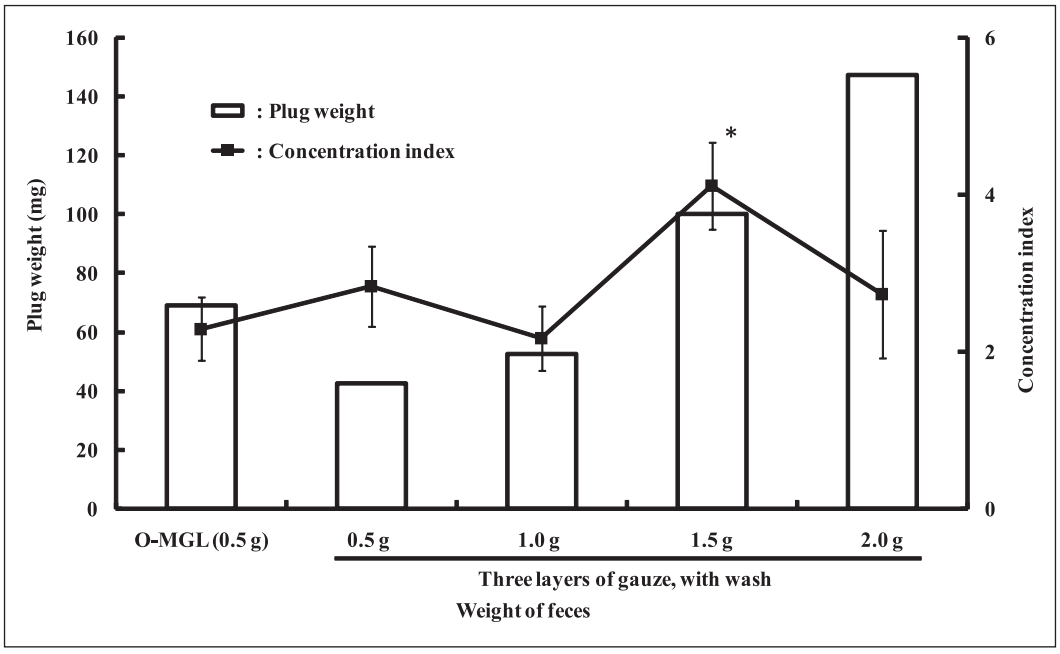

Figure 3. Effect of fecal weight on egg recovery
*Statistically significant compared with the O-MGL technique ($P < 0.05$).

Table 1. Comparison of the results of the O-MGL, M-MGL, Kit, KK, and DS techniques

Species	No. of positive (%)[a]					Mean no. of eggs detected				
	O-MGL*	M-MGL**	Kit	Kato-Katz	Direct	O-MGL	M-MGL	Kit	Kato-Katz	Direct
Ascaris lumbricoides	9 (18)	9 (18)	5 (22)	6 (12)	3 (13)	41	148	97	11	6
Trichuris trichiura	6 (12)	10 (20)	4 (17)	3 (6)	0 (0)	9	11	6	2	-
Hookworm	6 (12)	7 (14)	4 (17)	4 (8)	2 (9)	4	12	3	20	3
Clonorchis sinensis	2 (4)	2 (4)	0 (0)	0 (0)	0 (0)	2	9	-	-	-
Fasciolidae	1 (2)	1 (2)	0 (0)	0 (0)	0 (0)	1	3	-	-	-
Enterobius vermicularis	1 (2)	1 (2)	0 (0)	1 (2)	0 (0)	3	7	-	1	-
Recovery efficiency	32% (16/50)	40% (20/50)	39% (9/23)	24% (12/50)	22% (5/23)					
Sensitivity	76%	95%	90%	57%	50%					

[a]* versus **, $P > 0.05$.
In the case of the Kit and the direct smear (DS), only 23 faecal samples were used. For the rest of the techniques, 50 faecal samples were used.

samples were positive using at least one of the five techniques (only 23 samples were used for the comparative study of Kit and the DS techniques and 10 of those samples were positive for parasite). Therefore, the sensitivities of the M-MGL and the Kit techniques were 95% (20/21) and 90% (9/10), respectively. The mean number of *A. lumbricoides* eggs found in the M-MGL was 3.6-times higher than that in the O-MGL (Table 1).

DISCUSSION

Various modified MGL techniques have been reported by Young *et al.* (1979), Methanitikorn *et al.* (2003) and Parija *et al.*

(2003) . In these studies, the contribution to improvement of the O-MGL technique seemed to be limited because they compared only the positive rate of the MGL and the O-MGL techniques but not the individual process of the technique. We reevaluated the effect of the processes of the O-MGL (i.e., filtration, washing, pH, post-treatment, and weight of faeces) on the egg recovery for *A. lumbricoides* fertilized eggs. Egg recovery was evaluated using the CI, the importance of which is not the total number of recovered eggs included in the whole plug but the density of the eggs included in it.

The filtration of faecal suspensions using one layer of gauze has been recommended by Yoshimura *et al.* (1966) and Kamimura *et al.* (2000), two layers by Young *et al.* (1979), and three layers by another researcher. Although plug weight was reduced when we used more than three layers of gauze, the CI was also reduced because many more eggs were trapped on the gauze. The combination of using three layers plus washing improved the CI remarkably.

Post-treatment was carried out to reduce the plug weight and to facilitate observation. Re-filtration of the plug obtained from the O-MGL technique has been reported by Knight *et al.* (1976). They used only *Schistosoma mansoni*-positive samples, and the wire mesh used for the filtration had a large pore size (1800 µm). The general applicability of their technique therefore remains unknown. The plugs were also post-treated with gelatin solution and sucrose solution. Although the gelatin solution was adopted as a dispersing agent to reduce plug weight, positive results were not observed. Ritchie *et al.* (1960) added alcohol to the faecal suspension to reduce the specific gravity, and reported improvement in the recovery efficiency for eggs. In our preliminary experiment, we used a detergent substitute for alcohol but did not observe an effect (data not shown). We therefore used faecal suspensions with a high specific gravity by adding sucrose. We could reduce the plug weight because debris of low specific gravity was removed. Unfortunately, eggs were also removed simultaneously and the CI was not improved.

In relation to pH of the faecal solution, there is a report (Ritchie *et al.*, 1960) that the optimum pH for egg recovery varies according to the parasite. The optimum pH for the recovery of *A. lumbricoides* fertilized eggs is 10. Oshima *et al.* (1965) reported that the optimum pH for their technique was 4. Scum formation is explained by the adsorption of faecal materials (Vogel, 1952) or by emulsion formation (Oshima *et al.*, 1965).

In the M-MGL technique, we used three-times more faeces than for the O-MGL technique, but plug weight was only 1.5-times higher. Subsequently, the CI by the M-MGL technique was 1.8-times higher than that of the O-MGL technique. Although total processing time of the M-MGL is four minutes longer than that of the O-MGL, this technique exceeds other techniques in terms of sensitivity. The technique can therefore accurately reveal the prevalence in low endemic parasite areas.

Our result clearly revealed that the recovery efficiency, sensitivity, and mean number of eggs detected for the O-MGL technique were much lower than those of the M-MGL and the Kit techniques, though these differences were not statistically significant. This indicates that the usefulness of the O-MGL technique is not sufficient. The most likely reason for the decrease in the recovery efficiency of the O-MGL technique is a change in the composition of faeces in recent years. This has resulted from changing nutritional intake. Intake of animal fat, fat, and animal protein have increased 4–5 times compared with 60 years ago when the O-MGL technique was introduced (Ministry of Health, Labour and Welfare, Japan, 2006). It is quite natural to think that this change in eating habits will have affected faecal composition and, subsequently, the recovery efficiency of faecal examinations. Another reason for the low recovery efficiency seems to be related to the reduced intensity of the parasitic infection. Uga *et al.* (2005) reported that the mean

number of eggs per gram (EPG) of *A. lumbricoides*, *T. trichiura*, and hookworm obtained from an epidemiological survey in Hanoi, Vietnam, were only 880, 180, and 80, respectively. When these EPG values are evaluated according to the World Health Organization classification (1987), it is clear that the infection intensity of these parasites is very low. The mean number of eggs recovered from individual samples was higher in the M-MGL than in the other techniques. This indicates that the M-MGL can be applied to the field survey as an accurate faecal examination technique, particularly for the demonstration of prevalence in areas of low parasite endemicity.

Acknowledgements. We gratefully acknowledge the staff members of the Helminth Unit of the National Institute of Malariology, Parasitology, and Entomology (Hanoi, Vietnam) for their assistance during sample collection. This study was conducted as part of the core university program between Vietnam and Japan sponsored by the Japan Society for Promotion of Sciences (JSPS). Ethical approval for this study was obtained from the Medical Ethics Board of Kobe University (Approval Number 780).

REFERENCES

Ash, L.R. & Orihel, T.C. (1987). Parasite: A guide to laboratory procedures and identification. *American Society of Clinical Pathologists Press*, *Chicago* pp.23.

Chan, M.S. (1997). The global burden of intestinal nematode infections-Fifty years on. *Parasitology Today* **13**: 438–443.

Goodman, D., Haji, H.J., Bickle, Q.D., Stoltzfus, R.J., Tielsch, J.M., Ramsan, M., Savioli, L. & Albonico. (2007). A comparison of methods for detecting the eggs of *Ascaris*, *Trichuris*, and hookworm in infant stool, and the epidemiology of infection in Zanzibari infants. *The American Journal of Tropical Medicine and Hygiene* **76**: 725–731.

Hong, S.T., Choi, M.H., Kim, C.H., Chung, B.S. & Ji, Z. (2003). The Kato-Katz method is reliable for diagnosis of *Clonorchis sinensis* infection. *Diagnostic Microbiology and Infectious Disease* **47**: 345–347.

Kamimura, K., Iseki, M., Hirai, K. & Kimura, E. (2000). The spread of parasite. In: *Text of Parasitology* (in Japanese, Author's translation) pp. 2. *Bunkoudou*, *Tokyo*.

King, C.H., Sturrock, R.F., Kariuki, H.C. & Hamburger, J. (2006). Transmission control for schistosomiasis – why it matters now. *Trends in Parasitology* **12**: 575–582.

Knight, W.B., Hiatt, R.A., Cline, B.L. & Ritchie, L.S. (1976). A modification of the formol-ether concentration technique for increased sensitivity in detecting *Schistosoma mansoni* eggs. *The American Journal of Tropical Medicine and Hygiene* **25**: 818–823.

Lin, D.D., Liu, J.X., Liu, Y.M., Hu, F., Zhang, Y.Y., Xu, J.M., Li, J.Y., Ji, M.J., Bergquist, R., Wu, G.L. & Wu, H.W. (2008). Routine Kato-Katz technique underestimates the prevalence of *Schistosoma japonicum*: a case study in an endemic area of the People's Republic of China. *Parasitology International* **57**: 281–286.

Manson-Bahr, P.E.C. & Apted, F.I.C. (1982). *Manson's Tropical Diseases*, 18th edition pp. 181. English Language Book Society, London.

Methanitikorn, R., Sukontason, K., Sukontason, K.L. & Piangjai, S. (2003). Evaluation of the formalin-Tween concentration technique for parasitic detection. *Revista do Instituto de Medicina Tropical de Sao Paulo* **45**: 289–291.

Ministry of Health, Labour and Welfare, Japan. (2006). Change of nutritional intake. In: *The National Health and Nutrition Survey in Japan* pp 293. *Dai-Ichi Shuppan Pub. Co. LTD*, *Tokyo*.

Montresor, A., Crompton, D.W.T., Hall, A., Bundy, D.A.P. & Savioli, L. (1998). *Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level* pp 3. WHO CTD SIP.

Oshima, T., Kagei, N., Kihata, M., Fujino, N., Noguchi, H. & Fujioka, K. (1965). A new effective ether sedimentation procedure for the recovery of the eggs of *Clonorchis sinensis* and *Metagonimus yokogawai* in the stools. *Japanese Journal of Parasitology* **14**: 195–203.

Parija, S.C., Bhattacharya, S., Padhan, P. & Shivaprakash, M.R. (2003). Evaluation of formalin-acetone sedimentation in the concentration of stool for intestinal parasites. *Tropical Doctor* **33**: 163–164.

Ritchie, L.S. (1948). An ether sedimentation technique for routine stool examinations. *Bulletin. United States*, *Army Medical* Dept 8, 326.

Ritchie, L.S., Lin, S., Moon, A.P., Frick, L.P., Williams, J.E., Asakura, S. & Hishinuma, Y. (1960). The possible effects of pH and specific gravity on the ether-sedimentation procedure in concentrating eggs and cysts. *The American Journal of Tropical Medicine and Hygiene* **9**: 444–449.

Tada, I., Otomo, H., Kaneko, K. & Yamaguchi, T. (1987). Detecting techniques of parasite eggs in feces (in Japanese, Author's translation). In: *Essentials of Medical Parasitology* pp. 244. *Ishiyaku Pub. Inc.*, *Tokyo.*

Uga, S., Hoa, N.T., Thuan, le K., Noda, S. & Fujimaki, Y. (2005). Intestinal parasitic infections in schoolchildren in a suburban area of Hanoi, Vietnam. *The Southeast Asian Journal of Tropical Medicine and Public Health* **36**: 1407–1411.

Utzinger, J., Rinaldi, L., Lohourignon, L.K., Rohner, F., Zimmermann, M.B., Tschannen, A.B., N'goran, E.K. & Cringoli, G. (2007). FLOTAC: a new sensitive technique for the diagnosis of hookworm infections in humans. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **102**: 84–90.

Vogel, H. (1952). Leitfaden zur Untersuchung der tierischen Parasiten des Menschen und der Haustiere. (Reichenow-Vogel-Weyer) pp. 100. *Johan Ambrosius Barth Verlag*, *Leipzig.*

World Health Organization. (1987). Prevention and control of intestinal parasitic infections. *Report of a WHO Expert Committee*, *World Health Organization technical report series* pp. 12.

Yoshida, Y. (1985). Formalin ether sedimentation (MGL) technique. In: *Medical Zoology* pp. 21. *Nanzando Co. Lim.*

Yoshimura, H., Kamimura, K. & Kondo, K. (1966). The sedimentation technique. In: *Textbook of Parasitology* (in Japanese, Author's translation) pp. 227. *Bunkoudou*, *Tokyo.*

Young, K.H., Bullock, S.L., Melvin, D.M. & Spruill, C.L. (1979). Ethyl acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. *Journal of Clinical Microbiology* **10**: 852–853.

Yu, J.M., de Vlas, S.J., Jiang, Q.W. & Gryseels, B. (2007). Comparison of the Kato-Katz technique, hatching test and indirect hemagglutination assay (IHA) for the diagnosis of *Schistosoma japonicum* infection in China. *Parasitology International* **56**: 45–49.