THE FORMOL-ETHER CONCENTRATION TECHNIQUE FOR INTESTINAL PARASITES: COMPARING 0.1 N SODIUM HYDROXIDE WITH NORMAL SALINE PREPARATIONS

Jamsai Suwansaksri[1], Suwannee Nithiuthai[2], Viroj Wiwanitkit[3], Suphan Soogarun[4] and Pennapa Palatho[5]

[1] Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University;
[2]Division of Parasitology, Department of Pathology,Faculty of Veterinary Science, Chulalongkorn University;
[3]Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University;
[4]Department of Clinical Microscopy, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok;
[5]Jadtanin Hospital, Bangkok, Thailand

Abstract. The formol-ether method is a widely used technique for stool examination. We performed a comparative study of 0.1 N sodium hydroxide (NaOH) and normal saline preparation for the formol-ether technique in the detection of intestinal parasites. Of 30 parasite-containing stool samples, 22 (73%) were positive by 0.1 N NaOH and 18 (60%) were positive by normal saline. The detection rate of both preparations was not significantly different ($p>0.05$).

INTRODUCTION

Concentration techniques are performed in order to separate the parasites from fecal debris. Such techniques not only increase the number of parasites in the sediment but also unmask them, making them more visible by removing organic and inorganic debris. In most cases, diagnostic parasitology laboratories do not know the consistency of the stool, therefore full concentration and permanent staining are recommended (Foreyt, 1989). The formol-ether sedimentation technique is a concentration technique that is widely used.

The technique is appropriate for stool parasites, especially trematode eggs and protozoan cyst, in feces with a high fat content (Foreyt, 1989). Knight *et al* (1976) also reported more success in detecting light infections of *Schistosoma mansoni* in comparison with the Kato technique.

A number of modifications of the formol-ether technique have been developed in order to increase diagnostic sensitivity and ensure the effective use of resources (Knight *et al*, 1976; Akrum and van der Kuyp,1979). We performed a comparative study to consider the performance of 0.1 N sodium hydroxide (NaOH) and normal saline preparations in the formol-ether technique in the detection of intestinal parasites.

Correspondence : Suphan Soogarun, Department of Clinical Microscopy, Faculty of Allied Health Sciences, Chulalongkorn University , Bangkok 10330, Thailand.

MATERIALS AND METHODS

Stool specimens were collected from people who were living in an endemic area of Maung Rung village, Huai Thalaeng district, Nakhon Ratchasima Province on the assumption that they were harboring *Opisthorchis viverrini* eggs. All specimens were examined by simple smear with iodine and concentration technique using standard normal saline comparing to 0.1 N sodium hydroxide preparations. Two preparations were used for the formol-ether technique: one with 0.1 N sodium hydroxide (NaOH) and the other with normal saline. A total of 30 parasite-containing stool specimens were studied. The formol-ether technique used in this study was that described by Nithiuthai (1997). In summary: two to five grams of stool were used for each test. The stool was poured onto a double layer of wetted gauze taking special care to include any blood or mucus. Ten milliliters of a preparation of either normal saline or 0.1 N NaOH were mixed. The mixture was left for 1 hour before centrifugation for 5 minutes at 2,000 rpm. The supernatant was poured off and mixed with the reagent in step 2 again. Repeat centrifugation, at the same rate and for the same time, was conducted. The supernatant was poured off and 10ml of 0.1% formalin were added. The mixture was left for 10 minutes. Three milliliters of ether were added; the tube was closed and mixing took place for 30 seconds. The upper separated supernatant was poured off; the sediment was used for slide preparation.

Statistical analysis

The difference between the positive rate of detected parasite containing samples was compared by

Table 1
Result of 0.1 N NaOH and normal saline preparations.

Parasites	Preparation	
	0.1 N NaOH	Normal saline
Positive	22	18
OV + MF	11	8
OV + MF + HW	2	2
MF	8	5
MF + FB + HW	1	3
Negative	8	12

OV = *Opisthorchis viverrini*; MF = minute intestinal fluke; HW = hookworm , FB = *Fasciolopsis buski*

proportional test. Statistical significance was accorded to a p of 0.05.

RESULTS

Of 30 parasite-containing stool samples, 22 (73%) positive samples were derived from the 0.1 N NaOH preperation while 18 (60%) were derived from the normal saline preparation (Table 1). According to the study, the proportion of detection rate of both preparation was not different ($p>0.05$).

DISCUSSION

The diagnosis of parasitic infections in humans is challenging and requires the recognition of parasite stages based on size, morphology, color, and movement. Size and morphology are the major diagnostic parameters, (Foreyt, 1989). A concentration procedure should be performed as a routine part of a complete examination for parasites. Concentration permits the detection of organisms present in small numbers: these may be missed by using direct wet mounts. Organisms that can generally be identified using a concentration procedure include: helminth eggs and larvae; cysts of *Giardia lamblia, Entamoeba histolytica / Entamoeba dispar, Entamoeba coli, Endolimax nana, Blastocystis hominis, Iodamoeba butschii;* oocysts of *Isospora belli.*

It seems that 0.1 N NaOH preparation can provide a better detection rate (73%) than normal saline preparation (60%). However, no statistically significant difference could be shown. Furthermore, we found that some round worm eggs had degenerative surface changes that may have led to inaccurate identification. The turnaround time for the normal saline preparation was shorter. Therefore, both methods can be used in the preparation of formol-ether sedimentation technique in which 0.1 N NaOH will reduce stool debris that interfere in test system.

REFERENCES

Akrum S, van der Kuyp E. Formol-gasoline concentration technique for eggs and cyst. *Rec Bras Pesqui Med Biol* 1979;12:89-92.

Foreyt WJ. Diagnosis parasitology. *Vet Clin North Am Small Anim Pract* 1989;19:979-1000.

Knight WB, Hiatt RA, Cline BL, Ritchie LS. A modification of the formol-ether concentration technique for increased sensitivity in detecting *Schistosoma mansoni* eggs. *Am J Trop Med Hyg* 1976;25:818-23.

Nithiuthai S. Diagnostic techniques in veterinarian parasitology. Bangkok: Chulalongkorn University Press, 1997.