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Comparison of Formol-Ether, Direct Smear and Nigrosine Methylene Blue for the Diagnosis of Human Intestinal Parasites

Sheyin z[1*]., Bigwan El[1]., Galadima M[2].

[1]Department of Medical Laboratory science, University of Jos, Nigeria.
[2]Federal University of Technology, Minna, Nigeria.

E- mail for Correspondence: sheyinzakka@yahoo.com

ABSTRACT

In this study, we compared three methods of intestinal parasites diagnosis in order to come out with a simple, precise and affordable method. Intestinal parasitic infections are more prevalent in developing countries and their burden may even be more than that of bacterial infections. The Formol-ether concentration technique has been considered as a gold standard for the detection of most intestinal parasites; however, because of its low safety and hazardous impact, the need for a better and cheaper method is of paramount importance especially in this part of the globe. A total of 51 fecal samples were collected and each stool sample was examined by: Direct smear using saline and iodine preparation, Formol-ether concentration method and Nigrosine methylene blue concentration method. Of the 51 stool samples examined, 20(39.2%) were positive for parasitic infection. The sensitivity indicated that Nigrosine methylene blue has the highest percentage of 85%, followed by Formol ether with 75% and the Direct smear had the least with 65%. The sensitivity of the three methods indicated that Nigrosine methylene is good and is even better than the Formol-ether and should be preferred to the most celebrated Formol-ether and the conventional direct smear because even people with less technical expertise can use it more conveniently with good result.

Keywords: Paraisites, Formol-ether , Nigrosine methylene blue, Direct smear, Concentration.

INTRODUCTION

Organisms that live on other organism (host) and benefit from these organisms are called parasites (literally *para* - beside, *sitos* - food). It is obvious that the parasite can have no intention of doing harm to the host and that would not even be in the interest of the parasites itself. The damage is done accidentally especially if the number of the parasites becomes too large. Parasites may do harm to their host by absorbing food intended for the host example tape worm (Benesh, 2013). Blood or lymph could be sucked by parasites like hookworm. Parasites like ascaris feed on the tissue of the host causing wound through which infections may enter (Raina *et al*., 2013). Intestinal parasitic infections are among the major public and socio-economic concerns that adversely affect the well-being of the poor in developing countries (Christine and Christopher, 2000) and may even be more important than bacterial infections (Parameshwarappa *et al* .,2012). It has been estimated that *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* infect 1,450 million, 1,300 million and 1,050 million people worldwide, respectively, while schistosomiasis affects over 200 million people. *Entamoeba histolytica* and *Giardia lamblia* are also estimated to infect about 60 million and 200 million people worldwide, respectively (liza *et al*., 2011)

Considering the importance of the harmful effects of these parasites, there is need for proper diagnosis. The

examination of the faecal specimen for intestinal parasitic infections is enhanced by the use of concentration methods. The purpose for these methods is to increase the detection rates of the infections. Concentration of parasites ova or cysts from faeces may be accomplished in a number of ways. All floatation methods depend upon mixing fecal sample with a liquid, the specific gravity of which is greater than that of most of such forms yet less than the specific gravity of most of the fecal debris. Thus the parasites forms rises to the top of the flotation fluid by gravity. Various salts or chemicals which include: sucrose, glycerine, zinc sulphate, zinc acetate, sodium citrate, or magnesium sulphate have been used in flotation methods. The distortion of ova and cysts by the use of high salt concentration has been the limitation of the flotation method (Parameshwarappa *et al*.,2012).

Concentration of human intestinal parasites can also be achieved by sedimentation techniques which include Formol-ether sedimentation and formalin-ethyl acetate sedimentation. The difficulty in the storing, using and disposing of ether has made many laboratories to desire a concentration method that does not present the storage and disposal problems found with ether (Moges *et al*., 2010). In this study we compare the most popular Formol-ether concentration method, Nigrosine methylene blue concentration method and the conventional direct smear method.

METHODOLOGY

Study Area.

The study was conducted in Zaria using ECWA primary school Samaru, Zaria, Kaduna state, Nigeria and Sick Bay Ahmadu Bello University Zaria main campus.

Ethical Approval

Ethical approval was obtained from the ECWA primary school management and the Sick Bay hospital management. Consent were also obtained from the parents of the School children before sample were collected.

Sample Size

The numbers of stool samples collected were 51. Forty (40) samples were collected from ECWA primary school and 11 samples were collected from sick bay.

Direct Smear Examination

The formalinized stool samples were first filtered and two drops at different spots on the slide were taken. A drop of normal saline was dropped on one of the spot and 2% iodine to the other. Cover slips were applied and examined microscopically.

Formol-Ether Concentration Method

The sedimentation technique described by Truant *et al*. (1981) was followed. Approximately 5ml of formalinized stool specimen were strained through guaze into a centrifuge tube. The suspension was centrifuged and adjusted to obtain sediment of approximately 0.05ml. Distilled water was added to give a volume of 10ml. The suspension was then thoroughly mixed and centrifuged at 15000rpm for 20 minutes. The supernatant was decanted and formol-saline was added to make a volume of 10ml. A 3 ml amount of diethyl- ether was added. The tube was stopped and shaken vigorously for 30 seconds and the mixture centrifuged at 1500rpm for 2 minutes. The top three layers were decanted and saline and iodine preparations were made for microscopic examination.

Nigrosine Methylene Blue Concentration Method

The sedimentation technique described by Vinayak and Sehgal. (1976) was followed: Approximately 1g of human stool specimen was emulsified in 10 ml of distilled water. The emulsion was passed through a sieve. To the filtrate, two drops

Table 1. Number of types of parasites detected from each positive sample by each method

Positive specimen	Number of types of parasites diagnosed by:		
	Direct smear	Formol -ether	Nigrosine methylene blue
1	2	2	2
2	1	1	1
3	1	1	1
4	1	1	1
5	1	0	0
6	1	0	2
7	1	1	1
8	0	0	1
9	1	1	2
10	1	1	1
11	1	0	0
12	1	1	2
13	0	1	1
14	0	1	1
15	0	1	1
16	0	0	1
17	1	1	1
18	1	1	1
19	0	1	1
20	0	1	0
Total	13	15	17

Table 2. The total number of parasites ova or cyst detected by each of the methods

Name of parasite	Direct smear	Formol-ether	Nigrosine methylene blue
Ascaris	1	2	9
Hookworm	7	13	17
Enterobius	9	17	19
Trichuris	1	0	0
Strongyloides	5	1	6
Entamoeba coli	5	4	8
Entamoeba histolytica	3	2	5
Total	31(23.10%)	39(29.10%)	64(47.77%)

of a mixture of the stains which consisted of equal volume of 10% aqueous Nigrosine and 1% aqueous methylene blue were added. The tubes were then centrifuged at 2000rpm for 5 minutes. Most of the supernatant was discarded leaving only the volume of the fluid equal to that of the deposit. The mixture was gently homogenized and two drops at different spots on a slide were taken.

RESULT

Of the 51 stool samples examined, 20(39.2%) were positive for parasitic infection. The number of type of parasites in each positive sample detected by each of the methods was determined as shown in Table 1. The direct smear detected a total of 13 types, Formol-ether 15 types and Nigrosine methylene blue 17 types.

Table 2 shows the total number of parasites ova or cyst detected by each of the methods. The modified direct smear had 31 (23.10%), Formol-ether 39 (29.10%) and Nigrosine methylene blue had 64 (47.77).

Table 3. Sensitivity of the various diagnostic techniques in detecting ova or cysts of parasites

Parasite	No of positive samples	Direct smear	Formol -ether	Nigrosine methylene blue
Ascaris	4	0.25	0.5	2.5
Hookworm	6	1.70	2.17	2.80
Enterobius	4	2.50	4.25	4.75
Trichuris	1	1	0	0
Strongyloides	5	1	0.2	1.2
Entamoeba coli	3	1.67	1.33	2.67
Entamoeba histolytica	1	3	2	5
Total	20	1.55	1.95	3.2

Table 4. The sensitivity of the various diagnostic tests in parasitic diagnosis expressed in percentages

No of positive samples	Number of positive samples		
	Direct smear	Formol-ether	Nigrosine methylene blue
Total	13 (65%)	15 (75%)	17 (85%)

The sensitivity of the methods was calculated similar to that of Truant *et al.* (1981). For each method, sensitivity = total number of eggs or cysts in all positive sample**/**Total number of positive samples (Table 3). Nigrosine methylene blue had the highest 3.2, followed by formol-ether 1.95 while modified direct smear had the lowest 1.55.

The sensitivity of the 3 diagnostic methods in parasitic diagnosis was expressed in percentages as shown in Table 4. Nigrosine methylene blue has the highest percentage of 85%, followed by formol ether with 75% and the direct smear had the least with 65% (Table 4).

DISCUSSION

This study revealed that the Nigrosine methylene blue concentration method is as good as the formol-ether concentration method. In fact, it is more sensitive in detecting polyparasitism than formol-ether (Table 1). By comparison, the total number of parasites ova or cyst detected by each method revealed that the direct smear had 31 (23.10%), Formol-ether 39 (29.10%) and Nigrosine methylene blue had 64 (47.77) as shown in Table 2. The sensitivity by each method is shown in Table 3 where Nigrosine methylene blue had the highest 3.2, followed by Formol-ether 1.95 while modified direct smear had the lowest 1.55. The sensitivity of the 3 method was expressed in percentages as shown in Table 4. Nigrosine methylene blue has the highest percentage of 85%, followed by Formol ether with 75% and the Direct smear had the least with 65%. The similarity in sensitivity between Formol-ether and Nigrosine methylene blue suggested that we should choose the one that is simple and not time consuming for our routine laboratory work. The Negrosine methylene blue is simple in that it involves centrifugation only once. It is very good in our routine laboratories work where a large number of specimens are required to be examined within a short period of time. Vinayak and Sehgal (1976) had earlier reported that formol-ether concentration may not yield good results in the hand of an unskilled person as the procedure for breaking the fatty layer after the second centrifugation has to be done cautiously. The problem usually encountered with the conventional direct examination is that faecal debris may interfere with the examination. The Direct method of examination was improved upon by filtering the formalin fixed specimens before the slide preparations. It is important where we do not have centrifuge. The volume of solution required for the sedimentation techniques should also be considered. The formol-ether requires 10ml of formol-saline and 3ml of ether for a successful sedimentation and while only need 2 drops of a mixture of the stain for Nigrosine methylene blue concentration method.

Conclusion

The Nigrosine methylene blue is not commonly used even though it is a cheap and fast method for parasites diagnosis. Result from this work has shown it to have advantage over the much celebrated formol-ether concentration method.

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