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Comparison of Modified 1% Potassium Hydroxide Formol-Ether Concentration Technique with Direct Wet Mount Preparation and Standard Formol-Ether Concentration Technique for Detection of Parasites in Stool

Balakrishna J[1], Venkateswarlu S[2], Kusuma Bai[2], Hussain Saheb S[3].

[1] *Dept. of Microbiology, Santhiram Medical College, Nandyal,Andhrapradesh.*
[2] *Dept. of Microbiology, Fathima Inistitute of Medical Sciences, Kadapa ,Andhrapradesh*
[3] *Dept of Anatomy, JJM Medical College, Davangere, Karnataka.*

Abstract

Objective: - To compare the diagnostic yield of saline and iodine wet mount preparation with sedimentation techniques (formol-ether concentration technique (FECT) with physiological saline and modified formol-ether concentration technique with 1%potassium hydroxide) of fresh stool sample for diagnosis of human parasitic infections.

Method: - Direct wet mount stool slides were examined in saline and iodine preparation and concentration slides were prepared with FECT with physiological saline and FECT with 1% potassium hydroxide (KOH) under low power and high power.

Results:- Out of 200 stool samples,120(60%) were positive by direct wet mount preparation, 165 (83%) were positive by FECT with physiological saline and 164(82%) were positive by FECT with 1%KOH. The sensitivity of wet mount and modified 1%potassium hydroxide FECT were 73% and 99.3%, respectively. The positive agreement between FECT and wet mounts (84%) and modified 1%KOH formol-ether concentration techniques (99.7%) were good.

Conclusion: - Because of low sensitivity of wet mount preparation; our study conformed that the sedimentation techniques can be utilized for the diagnosis of parasitic infections in humans. The detection rate of FECT with physiological saline and FECT with 1% KOH was not significantly different ($p>0.05$).

INTRODUCTION

Human Intestinal parasitic infections persist as a global health problem (1) .Laboratory diagnosis of Intestinal parasitic infections depends on identification of intestinal parasites or morphological stages in their life cycle (ova, cyst, trophozoites, or larva) in stool samples (2). Wet mount preparations with saline & iodine is the conventional routine method to detect parasites in clinical samples .Wet mount preparations may fail to detect eggs, cysts or trophozoites when the sample contain parasites in small number. Concentration techniques not only separate fecal debris, it increases the number of parasites in the sediment and making them more visible by removing inorganic and organic debris (3). Formal-ether concentration technique is widely used, for detection of parasites especially trematode eggs and protozoan cyst, with a high content fat in the stool (4). For diagnostic purpose a number of modifications on formal-ether technique have been developed to increases the sensitivity of diagnostic and the effective use of resources (5, 1).

The present study compares with wet mount preparation and a modified 1%potassim hydroxide FECT against the standard procedure FECT method in field conditions (15).

MATERIALS AND METHODS

Two hundred stool samples were collected from patients who are suffering from persistent diarrhoea with abdominal pain, passage of worms, or allergic reactions cases at Santhiram General Hospital, Nandyal , Andhra Pradesh, India.

Clean, wide mouthed, dry bottles with lids were used to collect all stool samples. All faecal samples were examined by direct wet mount preparation with iodine and saline, and sedimentation techniques using slandered procedure FECT comparing to modified 1% potassium hydroxide FECT preparations. Three preparations were used from each sample one for direct wet mount, and second and third for formal-ether technique with normal saline and other with 1% KOH formol-ether concentration technique respectively. Wet mount preparation was carried out as following standard procedure (15). A drop of normal saline and iodine was put at both ends of a clean glass slide, with the help of wooden stick a minute portion of faeces was added in both drops and stirred to make uniform suspension. Coverslips was placed over each suspension separately, with out air bubbles and scanned for parasites for a minimum of 6 minutes before reporting negative. Standard procedure for FECT method was carried out as follows (6, 15).One to two grams of faecal

sample was used for each test. Faeces were mixed in 10ml of water and it was strained through two layers of gauze in a funnel. The filtered solution was centrifuged at 2000 rpm for 2min. Supernatant was discarded. The sediment was suspended in 10ml of physiological saline and again centrifuged, supernatant was discarded. 7ml of 10% formal-saline was added to the sediment allowed for stand for 30 min. Next 3ml of ether was added then tube was shaken vigorously and centrifuged at 2000 rpm for 2min. The supernatant three layers were poured off, the sediment was used for slide preparation. Modified Formal- ether concentration technique with 1% KOH was processed and prepared for microscopy with exactly the same procedure to FECT (15). But 1%KOH was used instead of physiological saline. Both methods were performed by experienced microbiologist. The results of FECT were correlated with the results of wet mount preparation and modified 1% KOH formol-ether concentration technique for all patients.

RESULTS

It was observed that FECT and modified 1% KOH formol-ether concentration technique proved to be very effective compare to the direct wet mount preparations for detection of parasites table 1. Of 200 stool samples, 120(60%) positive samples were derived form the wet mount preparation. 165(83%) were derived from the standard formol-ether concentration technique and 164 (82%) were derived from the modified 1% KOH formol-ether concentration technique preparation.

All specimens positive for parasites with the modified 1%KOH formol-ether concentration technique procedure were positive by standard FECT method. 1 of the 165 samples positive by the FECT method were negative by 1% potassium hydroxide FECT method , 45 of the 165 sample positive by the saline FECT method were negative by the wet mount preparation ,44 of the 164 sample positive by the 1% FECT method were negative by the wet mount preparation.
Modified 1% potassium hydroxide FECT and wet mount preparations are cross compared with the saline FECT in table 2. The sensitivities of wet mount preparation and modified 1% potassium hydroxide FECT were 73% and 99.3% respectively, and specifications were100% for each. The positive agreement between the FECT and wet mount preparations was 84% and between standard FECT and modified 1% potassium hydroxide FECT methods was 99.7%,indicating good agreement.

Table 1

Cross comparison of the modified 1% KOH formol-ether concentration technique and wet mount methods with standard FECT method.

Parasites	*Identification by Wet mount*	*Identification by standard FECT*	*Identification by modified1% KOH*
Entamoeba histolitica	39	55	55
Etamoeba coli	10	25	25
Giardia intestinalis	20	29	28
Ascaris	10	12	12
Hook worm	18	20	20
Hymenolepis nana	22	23	23
S.stercoralis	1	1	1

Table 2 –

Slide results by standard FECT method and Wet mount and modified 1% KOH formol-ether concentration technique methods.

	Standard FECT	*Positive*	*Negative*
Wet mount	Positive Negative	120 45	- 35
Modified 1%KOH FECT	Positive Negative	164 1	- 35

DISCUSSION

Various modified formol-ether concentration techniques have been reported (3,7) for diagnosis of human parasitic infections. The diagnosis of parasites in stool is establish by identification of ova and cysts by variety of techniques including direct wet mount preparations, sedimentation method, kato and FECT (8,9,10). Direct wet mount preparation has been widely used to demonstrate parasites in stool (11). However it lacks sensitivity. It could be repeat the examination 4-6 times (11, 12) this may be time consuming for laboratory persons and inconvenient for patients. How ever, additional use of FECT significantly (13, 14, 12). FECT permits the detections of organisms present even in small numbers: these may be missed by using direct wet mounts. It seems that modified 1% potassium hydroxide FECT can provide a better detection rate (82%) than wet mount preparation (60%). How ever no statically significant difference in detection rate between FECT and modified 1% potassium hydroxide FECT could be shown. Standard FECT and modified 1% KOH formol-ether concentration techniques showed greater sensitivity than wet mount preparation. Furthermore, we found that some round worm, eggs, had degenerative surface changes that may have led to inaccurate identification. Though turnaround time for the wet mount preparation was shorter, but parasites may not be detected in mild infections. However both sedimentation technique methods can be used in the preparations of formal -ether concentration techniques in mild parasitic infections, in which modified 1% potassium hydroxide FECT will reduce stool debris that interfere in test system.

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