Journal of the Egyptian Society of Parasitology, Vol. 51, No.1, April 2021
J. Egypt. Soc. Parasitol. (JESP), 51(1), 2021: 43 – 50
(Online: 2090-2549)

VALUE OF MCMASTER, MINI-FLOTAC, AND STANDARD TECHNIQUES FOR ASSESSMENT OF THE PREVALENCE OF INTESTINAL HELMINTHS AMONG SCHOOLCHILDREN IN MENOUFIA GOVERNORATE, EGYPT

By
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Abstract
Intestinal helminthiasis affects schoolchildren causing diseases. In this study, five copromicroscopic methods (3 traditional and 2 recent; Formaline-ether concentration method (FECM), Kato-Katz, direct smear, McMaster and mini-FLOTAC with two flotation solutions NaCl & ZnSO4 respectively) in addition to scotch adhesive tape method were used to detect helminthic infection in children. Hemoglobin percent and anthropometric measurements were also assessed. Out of 400 school children, 82 (20.5%) were infected; 75 single & 7 mixed infections. *Enterobius vermicularis* was the commonest one (11%) followed by *Hymenolepis nana* (9.75%) and the least one was *Schistosoma mansoni* (1.5%). The most sensitive method was mini-FLOTAC $ZnSO_4$ while the least one was direct smear, but without a significant difference. Scotch adhesive tape method had the best efficacy for *E. vermicularis* diagnosis. No significant difference was found for egg per gram (EPG) values of the counting techniques. *H. nana* was associated with anemia and underweight. Rural residence, unwashed fruits, and vegetables, unwashed hands before eating and after defecation were the risk factors. Sharing underwear and water canal contact were specifically associated with *E. vermicularis* and *S. mansoni* respectively. The better diagnostic performance were mini-FLOTAC and McMaster methods.
Keywords: Mini-FLOTAC, McMaster, Kato-Katz, FECM, Helminthes, Schoolchildren, Anemia.

Introduction

Intestinal parasites are among the commonest infections worldwide and cause health problems, particularly in children as anemia and adverse effects on their physical and cognitive growth (Bayoumy *et al,* 2018). Various risk factors were identified, including low health literacy, poor hygiene, intake of unwashed vegetables and fruit as well as water impurity (Hegazy *et al,* 2014).

Microscopic stool analysis is the primary tool to detect the intestinal parasites. Direct smear is the simplest and easiest procedure, but with low sensitivity (Abd El-Gaffar *et al,* 2018). Kato-Katz system, which is simple and low-cost, was recommended for laboratory and epideniological studies. But, its main drawback was the suboptimal vulnerability and misdiagnose of some helminthic eggs due to glycerol clearance effect (Lim *et al,* 2018). Formalin-ether concentration method (FECM) needed well-equiped lab. and was time-consuming (Kurt *et al,* 2012).

The McMaster technique proved to be simple, especially one in less-equipped and lab. technician, but with doubted efficiency in diangosing helminthic eggs (Levecke *et al,* 2011). Mini-FLOTAC is a simple, highly sensitive, and quantitative process with the advantage of using fresh or fixed samples (Barda *et al,* 2014).

The present study aimed to investigate prevalence and risk factors of intestinal helminthiasis among primary schoolchildren in Menoufia Governorate (Nile Delta, Egypt), to determine the helmi-nthiasis effect on children nutritional status, also to compare mini-FLOTAC and McMaster techniques with the ordinary stool exmination method.

Subjects and Methods

Study design and population: Cross-sectional study was achieved on 400 school children (6-12 years old) attending primary schools, during the period from February to May 2019. Children were selected using a multistage random sampling method. One city & one village were chosen from four Governorate districts. The first one was Ashmoon City & Kafr El-Sayed Village, the second was Berket El Sab City & Shentena El-Hagar Village, the third was El-Sadat City & Kafr Dawood Village and the fourth was El-Shohada City & Zawyet El-Bokaly Village.

Ethical consideration: This study was approved by the Committee of Research and Ethics, National Liver Institute, Menoufia University, achieved after endorsement from the MoH&P with written consent from children's guardians. A standardized questionnaire was used to assemble demographic data and information on possible risk factors.

Stools collection and examination: A sufficient portion of early morning feces was obtained from each child in a labeled, clean, leak-proof, and wide-mouth disposable container. Cellophane tape was sticked perianal and then adhered to a glass slide to detect *E. vermicularis.* Collected samples were tested microscopically by using 1- direct smear, a small portion of collected stools was put on a slide with a drop of saline (WHO, 1994). 2- Kato Katz, feces were pressed through a mesh screen to discard large elements and part of sieved sample was transferred to slide hole template, which was discarded after hole filling, remained sample (50mg) was covered with a piece of cellophane soaked in glycerol malachite green, and then number of egg per gram (EPG) feces was calculated by multiplying the recovered eggs number by 24 (Katz *et al,* 1972). 3- FECM, used for more effectiveness of centrifugal sedimentation to recover and concentrate helminths eggs from feces (WHO, 1991). 4- McMaster method, 2gm of feces were filtrated and homogenized with 30ml of saturated saline solution, then 1ml of suspension was added to each chamber and left for 3min. to float, eggs counted and multiplied by 50 to calculate EPG (Barda *et al,* 2014a). 5- Mini-FLOT AC, 2 flotation solutions were used; FS2 saturated sodium chloride with 1.20specific gravity and FS7, zinc sulfate with 1.35specific gravity. 2gm of stool were added to 2ml formalin (5%), homogenized, filtered, and added to either 36 ml FS2 or 46ml FS7. After 10min, eggs on reading discs were microscopically examined 10x & 40 x, EPG recorded as eggs/gm feces (Barda *et al,* 2014).

Anthropometric measurements: Bodyweight and height were measured using a scale to the nearest 0.1kg & 0.1cm respectively (Gibson, 2005). Differences from the median in standard deviation units were considered as values. Children classification as stunted, and underweight if height & weight for age were <2 standard deviation below WHO median for age and sex (Onis *et al,* 2007).

Hemoglobin levels: Hb was measured by DiaSpect^{Tm} Hemoglobin Analyzer (EKF Diagnostics, UK) that gave instant results from a finger prick. Children were categorized as anemic if Hb was <11.5g/dl (WHO, 2001).

Statistical analysis: Data were collected & computerized using SPSS (Statistical Package for Social Science) program for statistical analysis (version 20; Inc., Chicago. IL).

Results

Out of 400 children aged 6-12 years, 82 (20.5%) were infected; 75(18.75%) with one parasite and 7(1.75%) with mixed parasites. *E. vermicularis* was the commonest parasite (11%) followed by *H. nana* (9.75%) while *S. mansoni* was the least one (1.5%).

Sociodemographic data showed that younger age group (≤8 years) was significantly associated with parasitic infection, but, all *S. mansoni* cases were among older age group. Males were infected more than females, but without a significant difference. Children living in rural areas were significantly more infected than in urban areas. There was a significant difference in helminthic infection distribution, as Ashmoon showed the highest rate followed by El-Shohada, Berket El Sab, and El-Sadat was the lowest.

The habit of non-washing hands before eating and after defecation, eating outdoors, sharing underwear, water canal contact, and consumption of unwashed vegetables and fruits were significantly associated with helminthic infection. Difference between infected and non-infected children as to anemia and underweight was significant, but for stunted children, was not significant.

All the copromicroscopic data were considered as the golden standard diagnosis. The mini-FLOTAC ZnSO4 was highly sensitive follo-wed by mini-FLOTAC NaCl, McMaster, FECM, and Kato-Katz, but the least sensitive one was the direct smear. The negative predictive values of the different methods followed the same order. The Scotch adhesive tape method proved to be the best for *E. vermicularis* diagnosis compared to the used copromicroscopic method (44 vs 30 cases respectively).

The ROC curve showed that all used methods had significant AUC compared to combined results (Gr. 1). Regarding the number of eggs per gram of stool, mini-FLOTAC FS2 gave the highest EPG for *E. vermicularis* and *H. nana,* followed by mini-FLOTAC FS7, Mc-Master, and Kato-Katz. In *S. mansoni,* mini-FLOTAC FS7 gave the highest EPG followed by Kato-Katz (Gr. 2).

Details were given in tables (1, 2, 3, 4 & 5) and graphs (1 & 2)

Table 1: Intestinal helminths among schoolchildren:

Helminths recovered	Children number (%)
Single infection	
*E. vermicularis**	38 (9.5%)
H. nana	33 (8.25%)
S. mansoni	4 (1%)
Mixed infection	
E. vermicularis + H. nana*	5 (1.25%)
E. vermicularis + S. mansoni*	1 (0.25%)
H. nana + S. mansoni	1 (0.25%)

*: *E. vermicularis* detected by scotch adhesive tape method

Table 2: Prevalence of helminthic infections in correlation with sociodemographic data (age, sex, and residence):

Variable		Total examined (N=400)	Positive cases (No.=82)	χ2 test	P-value
Age	≤8 years	218 (54.5%)	58 (70.8%)	10.96	<0.001***
	>8 years	182 (45.5%)	24 (29.2%)		
Sex	Male	185 (46.3%)	44 (53.7%)	2.28	0.131
	Female	215 (53.7%)	38 (46.3%)		
Residence	Rural	200 (50%)	57 (69.5%)	15.71	<0.001***
	Urban	200 (50%)	25 (30.5%)		
Distribution	Ashmoon	100 (25%)	33 (40.2%)	17.36	<0.001***
	Berket El Sab	100 (25%)	15 (18.3%)		
	El-Sadat	100 (25%)	11 (13.4%)		
	El-Shohada	100 (25%)	23 (28.1%)		

*Significant (P < 0.05), **Highly significant (P < 0.01), ***Very highly significant (P < 0.001)

Table 3: Potential risk factors for intestinal helminthic infections among school children:

Variable		Positive (No.=82)	Negative (No.=164)	χ2 test	P-value	Odds Ratio (95% Confidence Interval)
Washing vegetables	Yes	53	138	11.99	<0.001***	0.3443
	No	29	26			(0.1858-0.6381)
Washing hand before food	Yes	34	99	7.86	0.005**	0.4651
	No	48	65			(0.2712-0.7976)
Washing hand after defecation	Yes	36	141	47.95	<0.001***	0.1277
	No	46	23			(0.0687-0.2373)
Sharing underwear	Yes	15	14	5.004	0.025*	2.3987
	No	67	150			(1.0960-5.2497)
Water canal contact	Yes	6	2	6.46	0.01*	6.3947
	No	76	162			(1.2613-32.422)
Eating food outdoors	Yes	23	19	10.47	0.0012**	2.9750
	No	59	145			(1.5089-5.8655)

Table 4: Effect of helminthic infection on nutritional status and hemoglobin level of infected children:

Variable		Single infection			Mixed infection (N=7)	Negative (N=164)	P value
		E. vermicularis (N=38)	*H. nana* (N=33)	*S. mansoni* (N=4)			
Height	average	37	32	4	7	162	P1: 0.5
	stunted	1	1	0	0	2	P2: 0.43
Weight	average	36	27	4	6	158	P1: 0.02*
	Under-weight	2	6	0	1	6	P2: 0.001**
Anemia	No	25	18	4	4	124	P1: 0.02*
	Yes	13	15	0	3	40	P2:0.003**

*Significant (P < 0.05), **Highly significant (P < 0.01), ***Very highly significant (P < 0.001), P1: Infected children with negative, P2: *H. nana* infected children with non-infected

Table 5: Sensitivity and negative predictive value of different stool examinations to detect *E. vermicularis, H. nana,* and *S. mansoni*

Variable	All helminths		*E. vermicularis* (n=30)		*H. nana* (n=39)		*S. mansoni* (n=6)		P-value
	S	NPV	S	NPV	S	NPV	S	NPV	
Direct smear	37.3%	87.4%	30%	94.6%	43.6%	94.3%	33.3%	98.9%	0.50
Kato-Katz	60%	91.6%	56.7%	96.6%	64.1%	96.3%	50%	99.2%	0.72
McMaster	81.3%	95.9%	86.7%	98.9%	89.7%	98.9%	0%	NA	<0.001***
FECM	77.3%	95%	80%	98.4%	76.9%	97.6%	66.7%	99.5%	0.77
Mini-FLOTAC FS2	90.7%	97.9%	100%	100%	97.4%	99.7%	0%	NA	<0.001***
Mini-FLOTAC FS7	94.7%	98.8%	96.7%	99.7%	92.3%	99.2%	100%	100%	0.60

*Significant (P < 0.05), **Highly significant (P < 0.01), ***Very highly significant (P < 0.001)
S: Sensitivity, NPV: Negative predictive value

Discussion

In the present study, the total intestinal helminths were 20.5% which agreed with Bayoumy *et al.* (2018) who recorded 22.5% in Beheira Governorate. *Enterobius vermicularis* was the most common helminth followed by *H. nana*, and the least one was *S. mansoni*. The high rate of *E. vermicularis* could be due to its highly infectious nature as the major transmission route is direct person-to-person contact or by autoinfection (Clark *et al,* 2014). The low *S. mansoni* rate was due to the success Egyptian National Bilharzial Control Program in Egypt, which reduced the percentage of infection (Fenwick, 2019).

In the present study, mini-FLOTAC ZnSO4 was the most sensitive procedure followed by the mini-FLOTAC NaCl, McMaster, FECM, Kato-Katz, and the least one was direct smear, which agreed with Cringoli (2006). In the present study, mini-FLOTAC ZnSO4 was more se-nsitive than mini-FLOTAC NaCl for *S. mansoni* detection, but mini-FLOTAC NaCl displayed better detection of *H. nana* and *E. vermicularis* (Catalano *et al,* 2019).

Alteration in prevalence of helminnths determined by different methods might be due to changes in t size of studied stool samples, concentration procedure and the flotation solutions used as their specific gravity in eggs flotation.

In the present study, the ROC curve identified the AUC for the copromicroscopic techniques showed significant AUC with mini-FLOTAC ZnSO4. Besides, NPV showed that mini-FLOTAC ZnSO4 method was the best technique used. These data agreed with Barda *et al.* (2013a). Also, when each method was compared with one another or with the combined data, the mini-FLOTAC NaCl and McMaster methods showed significantly better diagnostic value for both *E. vermicularis* and *H. nana* rather than *S. mansoni*. This agreed with Yimer *et al.* (2015) and Abd El-Gaffar *et al.* (2018).

In the present study, for *E. vermicularis* and *H. nana*, the mini-FLOTAC NaCl showed highest EPG followed by mini-FLOTAC ZnSO4, McMaster and the least one was of Kato-Katz. This agreed with Barda *et al.* (2014) and Adugna *et al.* (2017). But, with *S. mansoni*, the mini-FLOTAC ZnSO4 detected more EPG than Kato-Katz. This agreed with Barda *et al.* (2013b); Barda *et al.* (2014), and Coulibaly *et al.* (2016). Ng'etich *et al.* (2016) reported that helminthiasis was more evident in the hospitalized children samples with heavy infections rather than school children with mild to moderate infections.

In the present study, with *E. vermicularis* and *H. nana*, mini-FLOTAC NaCl was more dependable than the mini-FLOTAC ZnSO4, McMaster, and Kato-Katz methods, which agreed with Barda *et al.* (2014), but for *S. mansoni,* mini-FLOTAC ZnSO4 was better than FECM, or Kato-Katz, and direct smear.

In the present study, the Scotch tape for *E. vermicularis* eggs on perianal region was the best diagnostic technique. This agreed with Shoup (2001) and Kadir and Amin (2011).

In the present study, younger children (≤8 years) were significantly more infected, due to contagion, outdoor playing, crowded classroom, poor personal hygiene, or sharing personal tools. This agreed with Tigabu *et al.* (2019). Boys were more infected than girls, but without a significant difference, which agreed with Bayoumy *et al.* (2016); Liao *et al.* (2017) due to outdoors activities than females. Besides, children in rural areas were significantly more infected, which agreed with Mohammad *et al.* (2012) due to environmental pollution. Moreover, risk factors for helminthiasis were the non-washing hands before eating or after defecation, and consumption of unwashed vegetables and fruits. This agreed with Li *et al.* (2015); Salahi *et al.* (2019) and Mahmoudvand *et al.* (2020).

In the present study, there was a significant difference between infected and non-infected school children as to anemia and underweight, while for stunted children not significant, which agreed with Khalil *et al.* (1991) and Cabada *et al.* (2016)

In the present study, there was no association between *S. mansoni* and anemia which might be due to the low number of detected cases. But, Butler *et al.* (2012) found that *S. mansoni*-infected persons were anemic due to inflammation rather than iron deficiency.

The direct smear was simple, cheap and fast, but the McMaster and mini-FLOTAC methods were time consuming followed by Kato-Katz, and then FECM and being more complicated ones (Barda *et al,* 2014).

Conclusion

Enterobius vermicularis was the commonest parasite, followed by *H. nana,* but *S. mansoni* was present in some rural areas.

Mini-FLOTAC proved to be a valuable diagnostic technique. Awareness about parasitosis and nutritional status and anemia must be considered by Public Health Authorities.

Acknowledgments

The authors would like to thank the National Liver Institute, Menoufia University, Egypt for financial support and facilitating this work.

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Explanation of figures

Fig.1 (a-f): *H. nana* egg detected microscopically at 10x by direct smear (1a), Kato-Katz (1b), FECM (1c), McMaster (1d), mini-FLOTAC FS2 (1e), and mini-FLOTAC FS7 (1f).

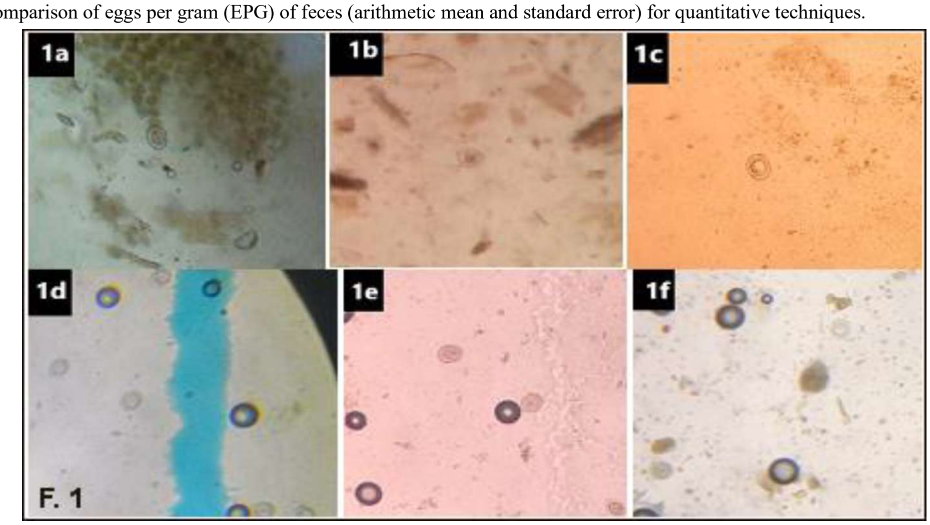
Fig.2 (a-f): *E. vermicularis* egg detected microscopically at 10x by direct smear (2a), Kato-Katz (2b), FECM (2c), McMaster (2d), mini-FLOTAC FS2 (2e), and mini-FLOTAC FS7 (2f).

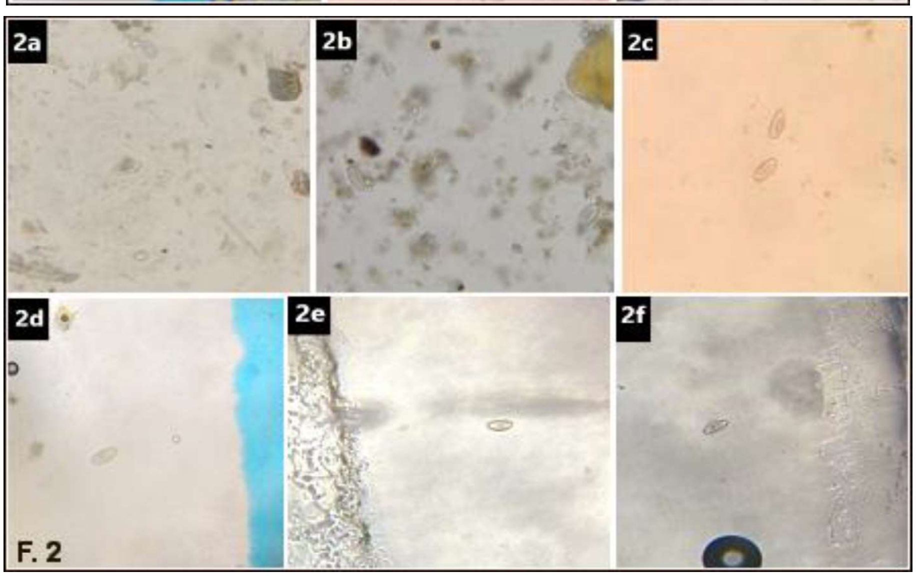
Fig. 3 (a-d): *S. mansoni* egg detected microscopically at 10x by direct smear (3a), Kato-Katz (3b), FECM (3c), and mini-FLOTAC FS7 (3d).

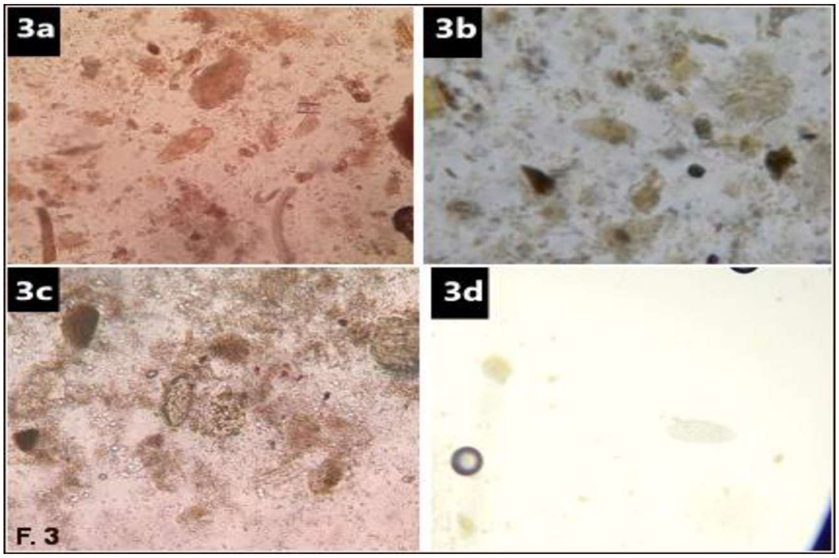
Fig.4 (a, b, & c): *E. vermicularis*, *H. nana*, & *S. mansoni* eggs detected microscopically at 40x by Kato-Katz, mini-FLOTAC FS2, & FECM respectively.

Gr.1: Receiving operation characteristics (ROC) curve analysis of different methods in detection of helminths.

Graph 2: Comparison of eggs per gram (EPG) of feces (arithmetic mean and standard error) for quantitative techniques.



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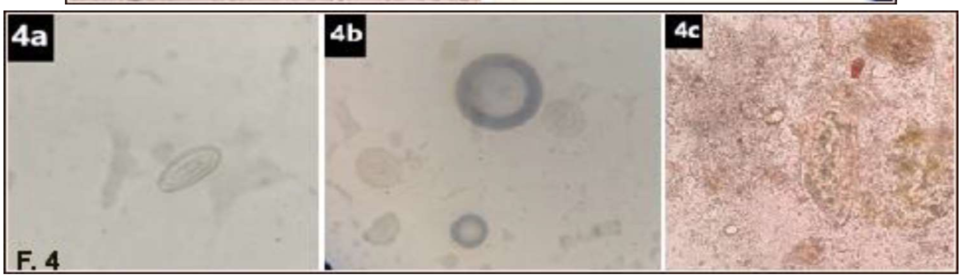
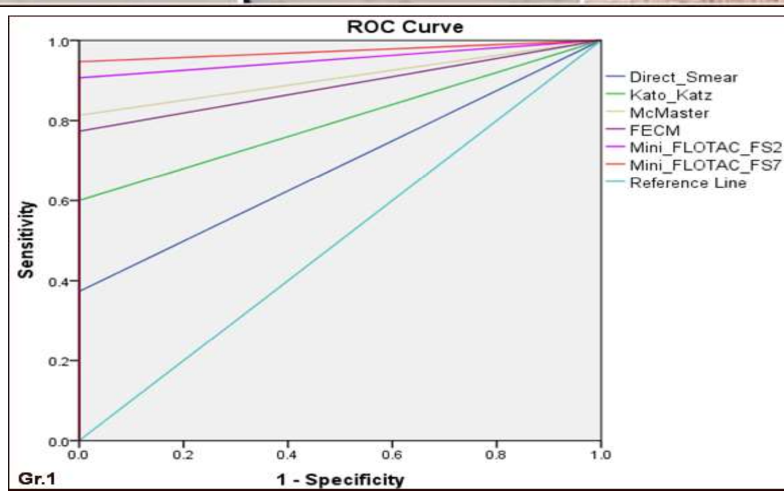
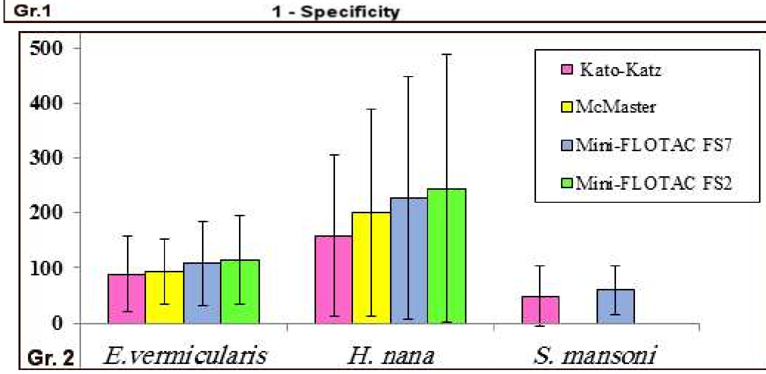
3d
F. 3


4a
4b
4c
F. 4


ROC Curve
Sensitivity
1 - Specificity
0.0
0.2
0.4
0.6
0.8
1.0
Direct_Smear
Kato_Katz
McMaster
FECM
Mini_FLOTAC_FS2
Mini_FLOTAC_FS7
Reference Line
Gr.1


500
400
300
200
100
0
Kato-Katz
McMaster
Mini-FLOTAC FS7
Mini-FLOTAC FS2
E.vermicularis
H. nana
S. mansoni
Gr. 2