

ADDIS ABABA UNIVERSITY

SCHOOL OF MEDICINE

DEPARTEMENT OF MICROBIOLOGY, IMMUNOLOGY, AND PARASITOLOGY



COMPARISON OF DIAGNOSTIC PERFORMANCE OF MINIPARASEP® SF FAECAL PARASITE CONCENTRATOR, KATO-KATZ THICK SMEAR AND MCMASTER TECHNIQUES FOR THE DIAGNOSIS OF INTESTINAL PARASITIC INFECTIONS AMONG WOSHA SOYAMA PRIMARY SCHOOL CHILDREN, WONDO GENET, SOUTHERN ETHIOPIA

BY

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GENET, SOUTHERN EHIOPA**

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LIST OF ABBREVIATIONS AND ACRONYMS

ALIPB	Aklilu Lemma Institute of Pathobiology
CI	Confidence Interval
FEC	Formol-ether concentration
EPG	Eggs per gram
FS	Flotation Solutions
IPIs	Intestinal parasitic infections
MDA	Mass drug administration
NPV	Negative predictive value
PPV	Positive predictive value
Rpm	Revolution per minute
SAF	Sodium acetate - acetic acid-formaldehyde
SF	Solvent free
SOPs	Standard Operational Procedures
SPSS	Statistical Package for the Social Sciences
Stata	a powerful statistical package that provides everything you need for statistics,data management and graphics
STHs	Soil-transmitted helminths
USD	United states dollar
WHO	World Health Organization

Operational Definition

Accuracy: the extent to which a given measurement agrees with the standard value for that measurement.

Sensitivity- is the probability that a truly infected individual will test positive.

Specificity- is the probability that a truly uninfected individual will test negative.

Reference standard test / gold standard' test- is a test that is used to identify which subjects are truly infected and which are uninfected against which the sensitivity and specificity of tests are evaluated.

Positive predictive value (PPV) - is the probability that those testing positive by the test are truly infected.

Negative predictive value (NPV) - the probability that those testing negative by the test are truly uninfected.

True positive- the individual has the condition and tests positive for the condition

True negative- the individual does not have the condition and tests negative for the condition

False positive- the individual does not have the condition but tests positive for the condition

False negative- the individual has the condition but tests negative for the condition

Combined result- results obtained by Miniparasep® SF fecal concentrator, Kato Katz and McMaster techniques: the union of positive results of the three methods as true positive and their intersection as true negative

Any species– all species (total) that were detected by the method under discussion

Abstract:-Background: Intestinal parasitic infections are responsible for considerable morbidity and mortality, especially among school-aged children. The diagnosis commonly relies on the detection of trophozoite, cyst, oocyst, parasites egg, or larvae in stool. However, infections often escape diagnosis due to inefficiency of diagnostic methods among others (like, inappropriate sampling and inadequate skill of laboratory personnel). So, the aim of this study was to evaluate or compare the diagnostic performance or operational characteristics of three different diagnostic techniques for the determination of the prevalence and intensity of intestinal parasitic infections in order to explore a better diagnostic method in the study area. **Method:** Institution based cross-sectional study was conducted in Wosha Soyama primary School, Wondo Genet area. Students were selected by systematic random sampling and stool samples were collected. Samples were screened for the detection and intensity of intestinal parasitic infection using Mini parasep ® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster egg counting technique. Data were entered in to Excel and analyzed with SPSS version 22 and Stata version 13.

RESULT: - Overall 86.1% (328/381) of samples were found positive for intestinal parasitic infection. Poly-parasitism were found in 54.1% (206/381) of the stool samples examined. The prevalence of intestinal parasites using the Mini parasep ® SF faecal parasite concentrator, Kato-Katz and McMaster were 77.7% (296/381), 68.8% (262/381) and 47.5% (181/381), respectively. The sensitivity, specificity, positive and negative predictive value of Mini parasep ® SF faecal parasite concentrator were 90.2%, 100%, 72.7% and 100%, for Kato-Katz thick smear 80.0%, 100%, 64% and 100% and for McMaster 55.2%, 100%, 57.6% and 100%, respectively.

CONCLUSION: Mini parasep ® SF faecal parasite concentrator detects many helminths including cysts of protozoan parasites than the two methods. It had low egg counting ability compared to the two methods, except higher mean egg count for *Hymenolopis nana* and Hook worm than Kato -Katz thick smear. Kato- Katz also showed better performance than McMaster except for Hookworm and *Hymenolopis nana*. McMaster showed better performance for Hookworm than both methods. In addition it had better performance than Kato- Katz for *Hymenolopis nana*. Kato-Katz also had more sensitivity and negative predictive value than McMaster. Moreover, Kato Katz showed better performance in assessing intensity of infection for most helminths detected than the two methods. Intestinal parasitic infections showed alarming prevalence and intensity of infection in study area. **Recommendation:** For mapping priority areas for control, monitoring and evaluation of programs impact, or for surveillance purpose and

routine diagnosis of intestinal parasites further evaluation of Mini parasep® SF relative to other methods and strength health promoting activities in this area is recommended.

Keywords: *Diagnostic performance, Mini parasep® SF, Kato-Katz, McMaster, Intestinal parasites, school children, Ethiopia*

1. INTRODUCTION

1.1 **Background:** Intestinal parasitic infestations (IPs) caused by helminths and protozoans continue to be a public health burden in many developing countries. They are a major concern particularly in sub-Saharan Africa (Harhay *et al.*, 2010). The parasitic infections have been described as constituting the greatest single worldwide cause of illness and disease (Mehraj *et al.*, 2008). It is estimated that some 3.5 billion people are affected, and that 800 million are children who are ill as a result of these infections (WHO, 2014). Among IPs more than 1.5 billion people are infected with soil-transmitted helminths infections (STHs) worldwide (WHO, 2013). IPs cause thousands of avoidable outpatient morbidity and mortality, especially in school-aged children. They are also the leading cause of malnutrition, iron deficiency anemia, malabsorption syndrome, intestinal obstruction, chronic dysentery, rectal prolapsed, respiratory complications, poor weight gain, mental retardation and other diseases (Bethony *et al.*, 2006).

So, reliable, sensitive and practical diagnostic tests are an essential tool in the control programs of these infectious diseases. A diagnostic test for an infectious agent can be used to demonstrate the presence or absence of infection, or to detect evidence of a previous infection. Demonstrating the presence of the infecting organism, or a surrogate marker of infection, is often crucial for effective clinical management and for selecting other appropriate disease control activities such as contact tracing.

To be useful, diagnostic methods must be accurate, simple and affordable for the population for which they are intended. They must also provide a result in time to institute effective control measures, particularly treatment (WHO, 2011). For some infections, early diagnosis and treatment can have an important role in preventing the development of long-term complications or in interrupting transmission of the infectious agent. In a broader context, diagnostic tests can have multiple uses, including: patient management, especially when clinical symptoms are not specific for a particular infection; screening for asymptomatic infections; surveillance; epidemiological studies (for example, rapid assessments of disease burden or outbreak investigations); evaluating the effectiveness of interventions, including verification of

elimination; and detecting infections with markers of drug resistance (McCarthy *et al.*, 2012, Solomon *et al.*, 2012).

Based on its uses, the requirements and expectations for a diagnostic tool in terms of technical performance, feasibility and costs change as control programs progress through different phases from initially high levels of infections to the confirmation of absence of infections. More precisely, during initial mapping to identify priority areas for control, when infection levels are typically highest, a diagnostic test with moderate sensitivity is acceptable, although the chosen tool needs to be easy to use, cost-effective and allow for the high-throughput screening of large populations (McCarthy *et al.*, 2012, Solomon *et al.*, 2012).

Since mapping data can also serve as a baseline for the monitoring and evaluation of programs impact, diagnostic tests must have sufficient performance to detect changes in the prevalence and intensity of infection (Solomon *et al.*, 2012). In later stages of programs, when infection prevalence and intensity have decreased significantly, more sensitive diagnostic tools are needed to establish an endpoint of treatment programs. If test sensitivity is insufficient at this point, light infections might be missed and this runs the risk of stopping control programs too early, before program endpoints have been achieved. Highly sensitive tests are also required for surveillance once treatment has been stopped to detect the potential re-occurrence of infections (McCarthy *et al.*, 2012, Solomon *et al.*, 2012). Finally, diagnostic tests play an important role in the assessment of treatment efficacy (Albonico *et al.*, 2012) and in patient management.

For the detection of human STH species, *A. lumbricoides*, *T. trichiura* and the Hookworms (*Necator americanus* and *Ancylostoma duodenale*), World Health Organization (WHO) recommends the use of Kato-Katz method in duplicate slides (WHO, 2002). Other commonly used methods include direct smear microscopy, formal-ether concentration (FEC), McMaster, FLOTAC and Mini- FLOTAC. All of these techniques rely on visual examination of a small sample of stool to determine the presence and number of STH eggs (WHO, 1994). Due to intra- and inter-sample variation in egg counts (Booth *et al.*, 2003; Krauth *et al.*, 2012), microscopy-based techniques can have differing sensitivities, especially in low transmission settings. Moreover, diagnostic methods vary considerably in the quantification of egg counts, which is

necessary to establish intensity of infection and to evaluate treatment effects (Albonicoet *et al.*, 2012; Knopp *et al.*, 2011; Leveckeet *et al.*, 2014).

When the performance of some diagnostic methods was compared for example: Kato-Katz thick smear method is more sensitive than McMaster technique for *A. lumbricoides* (84% vs 48%) and for other helminths (48% vs 43%) except for *H. nana* (49% vs 61%) (Barda *et al.*, 2014). Both McMaster and Kato-Katz methods are valid methods for monitoring large-scale treatment administration programs. Yet, the McMaster method seems more suitable for further standardization because of its robust multiplication factor, and allowing for simultaneous detection of all species of STH (Levecke *et al.*, 2011). As for egg counts, Kato-Katz thick smear has lower eggs count per gram of stool (EPG) for *H. nana* than Mini-FLOTAC and McMaster but has higher EPG for *A. lumbricoides*. The technique feasibility in terms of average time each method requires was calculated: Kato-Katz mean time was 48 min/sample, Mini-FLOTAC 13 min/sample and McMaster 7 min/sample. However, especially for Kato-Katz and Mini-FLOTAC, the mean time (min/sample) decreased significantly when processing multiple samples (Barda *et al.*, 2014).

The sensitivities of the Kato-Katz and Mini-FLOTAC techniques were comparable and in high intensity settings both techniques provide a practical and reliable diagnostic method (Nikolay *et al.*, 2014, Barda *et al.*, 2013). A particular advantage of the Kato-Katz method is the ability to simultaneously detect STH and *schistosome* species at low cost. The widely used double slide Kato-Katz method had a sensitivity of 74–95% for the three STH species at high infection intensity; however sensitivity dropped to 53–80% in low intensity settings, being lowest for Hookworm and *A. lumbricoides* (Nikolay *et al.*, 2014).

FLOTAC had a higher sensitivity than the Kato–Katz method for Hookworm diagnosis and the sensitivities of PCR and the Kato–Katz method were equal for this parasite (Knopp *et al.*, 2014). The high sensitivity of a single FLOTAC examination for diagnosing common STH infections has been also confirmed, but faecal egg counts were consistently lower when compared to the Kato-Katz method. Importantly, it was also shown that the FLOTAC method holds promise for the detection of *S. mansoni* eggs, particularly in well-homogenized stool samples after

preservation in Sodium acetate - acetic acid-formaldehyde (SAF) for at least 10 days (Glinz *et al.*, 2010).

Both Kato-Katz technique and FEC methods showed a better sensitivity than the traditional direct wet mount method (Endris *et al.*, 2013). Therefore, the employment of FEC techniques as a confirmatory test in routine laboratory examination of stool and Kato-Katz in epidemiological studies will significantly aid in accurate determination and management of parasitic infections in the community (Endris *et al.*, 2013). But in other study it was also shown that even though the Kato-Katz thick smear is the most recommended method for epidemiological study of *S. mansoni*, it has shown lower sensitivity than concentration technique in the diagnosis of helminths (Taye, 2014).

Kato-Katz technique can perform with reasonable accuracy with one day's stool collection for *A. lumbricoides* and *T. trichiura*. Low sensitivity of the Kato-Katz for detection of Hookworm infection also shown as it may be related to rapid degeneration of delicate Hookworm eggs with time (Tarafder *et al.*, 2010). But it is a highly sensitive technique for the detection and quantification of Hookworm infection in human parasitological surveys than simple sodium nitrate flotation technique (SNF). SNF offers resource-poor communities logistic feasible, freely available and cost-effective option to monitor the success of Hookworm control programs. SNF holds promise for the detection of human Hookworm and potentially other STH infections and may become an essential tool for patient management, monitoring of helminth control programs and anthelmintic drug efficacy studies in areas with no access to the commercially produced parasitological flotation devices (Inpankaew *et al.*, 2014).

Mini Parasep®SF method can be used in place of the modified formol-ether method for the laboratory diagnosis of intestinal parasitism especially in endemic areas. The recovery of intestinal parasites (diagnostic stages) by the two methods was comparable, although Mini Parasep®SF is preferred due to the inherent advantages. The sensitivity, specificity, positive and negative predictive values for modified formol-ether and Mini Parasep®SF techniques respectively are as follows; sensitivity (60% vs 93%), specificity (80% vs 96%), positive predictive values (57.4% vs 91.3%) and negative predictive values (81.7% vs 96.8%)(Ikeh

&Elujola ,2015). But in another study it was reported as FEC technique was more efficient in detecting helminth infections while the Mini Parasep performed better in detecting protozoan infection (Useh et al., 2011). The Mini Parasep was simple, user-friendly and involves working in an enclosed system with little or no danger of acquiring infection while the FEC technique is more labor intensive with potent danger of acquiring infection and outbreak of fire because of the use of ether in an “open” system. It was also confirmed as Mini Parasep is efficient for the detection of intestinal parasites in endemic areas (Useh et al., 2011).

Similarly evaluation of Parasep® SF, ether and ethyl acetate free faecal concentrators with Mini parasep ® which uses ether or ethyl acetate was shown as Parasep® SF is generally accepted very favorably by the laboratory staff. No problems in handling the device were encountered and training its use took a matter of minutes. The lack of fat globules in the deposit obtained using Parasep® SF compared to methods using ethyl acetate enabled better interpretation of protozoa cyst morphology and also gave a better deposit without the dense sticky fluid content frequently seen in other methods. Finally it was also indicated that the Parasep® SF faecal parasite concentrator can be satisfactorily used as a replacement for methods which rely on a solvent extraction process and will give enhanced recovery and safety features to the procedure which uses Parasep® with solvent (Moody *et al.*,2013).

Taking the advantages and disadvantages of the different tests together it was necessitated to evaluate or compare the diagnostic performance or operational characteristics of these three different diagnostic techniques for the determination of the prevalence and intensity of intestinal parasitic infections, for mapping priority areas for control, for monitoring and evaluation of programs impact, or for surveillance purpose as well as for the routine diagnosis of intestinal parasites. Thus, the aim of this study was to compare diagnostic performance of Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster techniques for the diagnosis of intestinal parasitic infections among Wosha Soyama primary school children in Wondo Genet, southern Ethiopia.

1.2. Statement of the problem

For the diagnosis of each intestinal parasite and for the evaluation of any new diagnostic method there is no gold standard method which is both sensitive and specific that can be used for all species. So, evaluation of any new diagnostic method needs taking the combined results of the methods compared together as gold standard. Due to this there is a difference in the performance of one diagnostic method when it compared with different methods each time.

Some diagnostic tests are sensitive but not specific enough or vice versa. Some are simple, but others are difficult to perform. Some are costly and time consuming but other can be cheap but have no good performance. Some diagnostic methods have robust multiplication factors (Levecke *et al.*, 2011) while the others lack. Some needs different diagnosing time from single sample. For example, Kato - Katz need different diagnosing time for different intestinal parasites (Bergquist *et al.*, 2009). Others need additional infrastructure and not used in filed setting. Some methods require preservatives which may change the morphology of parasites (Glinz *et al.*, 2010). So they are not equally used for routine diagnosis, surveillance, drug efficacy test and for the study of intensity of infection.

Since the Ridley-Allen modified formol ether sedimentation technique was first developed and recent years commercial concentration tubes such as Parasep®, Apacor Ltd, Wokingham, United Kingdom have evolved to form the mainstay of a modern microbiology laboratory. The problem with fat cells occluding the parasites however has remained, and necessitates the addition of a lipid solvent, in the form of ether or ethyl acetate (Moody *et al.*, 2013). The lipid solvents used (ether or ethyl acetate) are highly flammable and irritants, requiring special storage and disposal conditions (Moody *et al.*, 2013).

The results from this study elucidated the diagnostic performance of Mini parasep ® SF faecal parasite concentrator, Kato - Katz thick smear and McMaster egg counting techniques. It also provided information about the best methods to use from the three techniques for intestinal parasites diagnosis. Finally, evaluation of these three diagnostic approaches was needed in the search for better diagnostic techniques for monitoring of progressing helminths control programs, confirmation of elimination, or surveillance of disease recrudescence.

1.3. Significance of the study

This study has many importances among these: it gives information on the most effective method for the diagnosis of intestinal parasitic infections among Mini parasep ® SF faecal parasite concentrator, Kato - Katz thick smear and McMaster techniques. It also gives information on the best diagnostic methods to be used for surveillance; epidemiological studies and evaluating the effectiveness of interventions, including verification of elimination. It provided information on the current prevalence and intensity of intestinal parasite infections in the study area.

It could be used as source of information by concerned bodies who are responsible for health promotion activities.

2. OBJECTIVE

2.1. General objective

- To compare diagnostic performance of Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster techniques in the diagnosis of intestinal parasitic infections and to assess the situation of intestinal parasitic infection in the study area

2.2. Specific objectives

- To assess the performance of Mini Parasep® SF faecal parasite concentrator for intestinal parasite diagnosis
- To assess the performance of Kato-Katz thick smear for intestinal parasite diagnosis
- To evaluate the diagnostic performance of McMaster technique for intestinal parasite
- To compare the sensitivity, specificity, PPV and NPV of the three tests
- To determine the prevalence of intestinal parasites infection in the study area
- To determine the intensity of intestinal parasitic infection in the study area

3. METHODS AND MATERIALS

3.1. Study Area and Study Design

A cross-sectional institutional-based study was conducted from December 1, 2014 to July 30, 2015 to compare the diagnostic performance of Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster technique for the diagnosis of intestinal parasitic infections among school-age children in Wosha Soyama Primary School which is found in Wondo Genet Town. **Wondo Genet** is a resort town in Ethiopia, located southeast of Shashemene in the Sidama Zone of the Southern Nations, Nationalities and peoples' Region, with a latitude and longitude of 7°1'N38°35'E / 7.017°N 38.583°E and an elevation of 1723 meters at 264 km south from Addis Ababa. According to Wondo Genet District's report in 2007 Ethiopian calendar the total population of Wondo Genet Town was 16,936 out of which 10,014 were females and 6922 were males. In the town there are two elementary school, five kindergartens, one health centre and one health post. During the study time a total of 3550 students enrolled from grade 1-8 in Wosha Soyama Primary School out of which females were 1799 and males 1751.

3.2. Source Population and Study Population

From all children living in Wondo Genet area during the study period, a total of 381 children enrolled at Wosha Soyama Primary School during the study period were participated.

3.3. Sample size

Sample size was calculated using single population proportion formula estimate considering 5% level of the significances and 50% prevalence at 95% confidence interval (CI).

Sampling technique: systematic random Sampling technique was employed to select the study students from grade 1-8. First proportional allocation of sample size was done based on size of source population (N=3550), required sample size (n=381) and total students of each class. Then after k was obtained for each class ($k = \text{population size}/\text{sample size}$), finally study participants were selected by random start between 1 and k^{th} element and then proceeds with the selection of every k^{th} element from then onwards.

Exclusion criteria

All primary school children who were treated with anti-helminths or anti-protozoan drugs or received mass drug administration (MDA) within the past four months were excluded from the study.

3.4. Data Collection

Stool sample collection

All consented students were supplied with small plastic sheet and applicator stick, and requested to bring about 5 grams of stool sample. On submission of stool samples, details on age and sex were recorded for each child.

Stool examination

The stool specimens were examined for intestinal parasites with Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster technique. Kato - Katz thick smears were examined within 30 minutes for Hookworm and after one hour up to 24 hours for other intestinal parasites in the study school and Wosha Soyama Health center respectively. By Mini Parasep® SF faecal parasite concentrator in Arsi University collage of Health Sciences Laboratory Department within one week of collection (stool preserved in 10% formalin) and with McMaster technique in ALIPB within two weeks of sample collection (also preserved in 10% formalin).

3.5. Data processing and analysis

Data entry and analysis were done using Excel and SPSS version 22.0 statistical software for descriptive statistics. Prevalence, sensitivity, specificity, positive and negative predictive values were analysed by Stata version 13 and kappa Estimator was employed to determine the strength of agreement of each methods with the combined result. Kappa values were interpreted as follows from 0.01–0.20 slight agreement, from 0.21– 0.40 fair agreement, 0.41–0.60 moderate agreement, 0.61–0.80 substantial agreement and 0.81–0.99 perfect agreement (Landis and Koch, 1977).

3.6. Data Quality Control

Specimen were collected, processed and examined following manufacturer instruction for Mini Parasep® SF faecal parasite concentrator and standard operating procedures (SOPs) for Kato

Katz thick smear and McMaster egg counting technique (see annex III). Stool samples were examined by two laboratory technologists. Discordant results were read by more experienced third laboratory technologist. Finally the data was analysed and interpreted accordingly.

3.7. Ethical Considerations

Ethical clearance was obtained from Department Ethics and Review Committee, Department of Microbiology, Immunology and Parasitology, collage of health sciences, Addis Ababa University. Permission was obtained from local administration and school director and teachers. Informed oral consent was obtained from the study participants and written consent from guardians. All study participants found positive for parasites were treated with single dose of 400mg Albendazole for *A. lumbricoides*, *T. trichiura*, *E. vermicularis*, *H.nana*, *Taenia* species and Hookworm and withpraziquantel 40 mg/kg body weight for *S. mansoni* by health extension workers of Wondo Genet town.

4.0. RESULT AND DISCUSSION

4.1. Result

Three hundred eighty one (n=381) students were enrolled by giving stool sample for intestinal parasites examination by Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear, and McMaster techniques, out of which 193 (50.7%) were females. The age range was between 5 and 18 years old with mean 9.67 years and Standard Deviation of 2.594.

From the total enrolled study subjects 86.1% (328/381) were found to be infected by one or more intestinal parasites (Table-1). *A. lumbricoides*, *T. trichiura*, *S. mansoni*, *H. nana*, Hookworm, *E. vermicularis*, *E. histolytica/dispar*, *G. lamblia* and *Taenia* species were the species detected. *T. trichiura* was the leading helminth detected followed by *A. lumbricoides* and *S. mansoni* and the least prevalent intestinal parasite detected was *E. vermicularis* (Table-1). By taking the combined result as a gold standard the prevalence of each detected parasites were *A. lumbricoides* 50.4%, *T. trichiura* 53.8%, *S. mansoni* 34.1%, *H. nana* 12.6%, Hookworm 14.7%, *E. vermicularis* 0.8 %, *E. histolytica/dispar* 2.1%, *G. lamblia* 1.1% and *Taenia* species 6.3% (Table-1).

The prevalence of intestinal parasites detected by Mini Parasep® SF faecal parasite concentrator was 77.7% (296/381) (Table-1). It detected 50 samples that were negative by Kato-Katz thick smear and 121 samples that were negative by McMaster egg counting technique but positive by the combined results of the three methods. The major prevalent parasites detected by this method were *A. lumbricoides* 46.7% (178/381), *T. trichiura* 37.5% (143/381) and followed by *S. mansoni* 31.8% (121/381) (Table-1).

The prevalence of intestinal parasites detected by Kato-Katz thick smear was 68.8% (262/381) (Table-1). It detected 99 samples that were negative by McMaster and 20 samples that were negative by Mini parasep ® SF faecal parasite concentrator. The major prevalent parasites detected by this method were *T. trichiura* 44.4% (169/381), *A. lumbricoides* 31.2% (119/381), and followed by *S. mansoni* 15.2% (58/381).

The prevalence of intestinal parasites detected by McMaster egg counting technique was also 47.5% (181/381) (Table-1). It also detected 12 samples that were negative by Mini parasep ® SF faecal parasite concentrator and 20 sample that were negative by Kato-Katz thick smear. The

major prevalent parasites detected by this methods were *A. lumbricoides* 29.4% (112/381), *T. trichiura* 16.3 % (62/381), and followed by *H. nana* 8.9% (34/381).

Table- 1. Prevalence of intestinal parasites based on Mini parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster egg counting techniques among children in Wosha Soyama primary school , March – April 2015. (n = 381)

Parasite species detected	Prevalence detected by each methods								
	Mini Parasep® SF faecal		Kato-Katz thick smear		McMaster technique		Combined result		P-Value
	n	%	n	%	n	%	n	%	
<i>A. lumbricoides</i>	178	46.7	119	31.2	112	29.4	192	50.4	<0.001
Hookworm	24	6.3	7	1.8	33	8.7	56	14.7	<0.001
<i>S. mansoni</i>	121	31.8	58	15.2	NPF		130	34.1	NA
<i>T. trichiura</i>	143	37.5	169	44.4	62	16.3	250	53.8	<0.001
<i>H. nana</i>	43	11.3	9	2.4	34	8.9	48	12.6	<0.001
<i>Taenia Spp</i>	23	5.5	7	1.8	NPF*		24	6.3	NA
<i>G. lamblia</i>	4	1.1	NPF		NPF		4	1.1	NA
<i>E. vermicularis</i>	3	0.8	2	0.5	NPF		3	0.8	NA
<i>E. istolytica/dispar</i>	8	3.9	NPF		NPF		8	2.1	NA
Any intestinal parasite	296	77.7	262	68.8	181	47.5	328	86.1	<0.001

* NPF= No parasite found by the specific method, n= number positive

NA= not available

Poly-Parasitism detected in the area

More poly-parasitism 206/381 (54.1%) and only 123/381(31.5%) single infections were detected in the study area. 29.1% students had double infection, 17.8% had triple infection, 6.3% had quadruple infection and only 0.8% students were infected by five species (Fig.1).

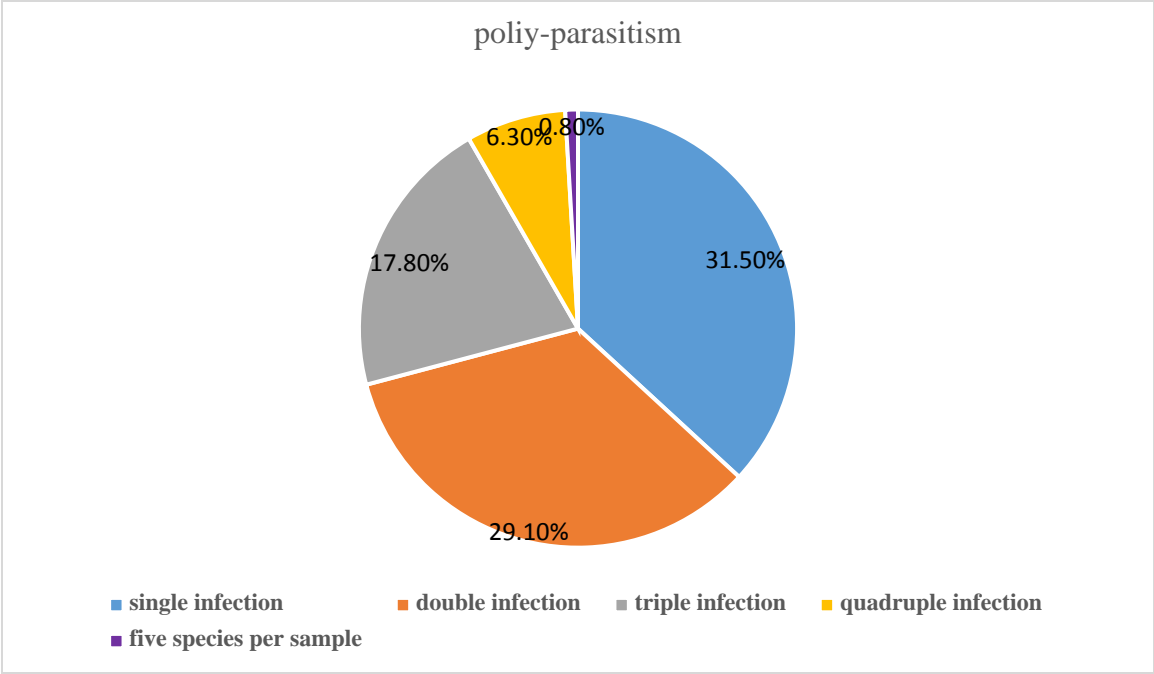


Figure-1. poly-parasitism detected in Wosha Soyama Primary school children, Wondo Gent, southern Ethiopia, from March-April 2015

Intensity of helminths infection

The intensity of infection was calculated based on WHO guide line for *A. lumbricoides*, *T. trichiura*, *S. mansoni*, and Hookworm (WHO, 2002). Intensity of infection was not calculated for

Table-2. Intensity of intestinal helminths infection in 1g stool depending on the combined results of the three methods among Wosha Soyama primary school children, March – April 2015. (n=381)

Helminths	Level of intensity of infection (EPG)			Level of intensity of infection according to WHO classification(EPG)		
	light	moderate	heavy	light	moderate	Heavy
<i>A. lumbricoides</i>	101(26.2%)	51(13.4%)	40(10.5%)	1-4999	5,000-49,999	≥ 50,000
<i>T.trichiura</i>	59(15.5%)	112(29.4)	33(8.7%)	1-999	1,000-9,999	≥10,000
Hookworm	56(14.7%)	0	0	1-1999	2,000-3,999	≥4,000
<i>S.mansoni</i>	67(17.6%)	6(1.6%)	61(16%)	1-99	100-399	≥400

EPG - eggs per gram of stool

rare parasites (*E. vermicularis*, *Taenia* spp. and *H. nana*). The intensity of infection after converting the amount of eggs counted in 0.3g of stool by Mini Parasep® SF faecal parasite concentrator, in 0.0417g of stool by Kato- Katz thick smear and in 2g of stool by McMaster in to eggs per one gram of stool (EPG) was shown in table-2.

Average egg counted by Mini Parasep® SF faecal parasite concentrator, Kato- Katz thick smear and McMaster for *A. lumbricoides* was 98, 941 & 564, for *T. trichiura* was 6, 105, & 42, for *H. nanawas* 34, 0.3, & 94 , and for Hookworm was 0.77, 0.44, & 8.1 respectively. For *S. mansoni* was 5 & 28; for *E. vermicularis* was 0.03, & 0.12; and for *Taenia* spp was 1.5 & 86 by Mini Parasep® SF faecal parasite concentrator and Kato- Katz thick smear respectively (Table -3).

Table- 3. Average egg counted of helminth parasites based on mini parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster egg counting techniques among children in Wosha Soyama primary school , March – April 2015. (n = 381)

Helminth species	Mean egg per gram of stool		
	Mini Parasep® SF faecal parasite concentrator	Kato-Katz	McMaster
<i>A. lumbricoides</i>	98	641	564
<i>T. trichiura</i>	6	105	42
<i>S. mansoni</i>	5	28	NPF*
<i>H. nana</i>	34	0.3	94
Hookworm	0.7	0.4	8
<i>E. vemicularis</i>	0.03	0.12	NPF
<i>Taenia species</i>	1.5	86	NPF

* NPF= No parasite found by the specific method

Sensitivity, specificity, NPV and PPV

Stool diagnosis results based on the combined Mini Parasep® SF, McMaster and Kato-Katz thick smear techniques were used as Gold standard to estimate sensitivity, specificity, negative

predictive value and positive predictive value of each methods in detecting the different helminths species (Table-4). Mini Parasep® SF technique was found more sensitive in identifying children who were infected with *A. lumbricoides* and *H. nana* and better in predicting children who were negative for these parasite species than the Kato-Katz thick smear and the McMaster techniques. Kato-Katz thick smear method was more sensitive in identifying children who were infected with *T. trichiura* and better in in predicting children who were negative for this parasite than the Mini Parasep® SF and McMaster techniques. The McMaster technique had better sensitive and negative predictive value in diagnosing Hookworm infection compared to the Mini Parasep® SF and the Kato-Katz thick smear methods. The Mini Parasep® SF was more sensitive than the Kato Katz technique in detecting *S. mansoni* infection. All children were negative for *S. mansoni* infection based on results of the McMaster technique. However, specificity and positive predictive values of detecting the different intestinal helminths parasites was similar (100%) in all the three tests.

Table- 4. Sensitivity, specificity, NPV and PPV of Mini parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster egg counting techniques in diagnosing intestinal parasite infection among school children in Wosha Soyama primary school children, Wondo Genet southern Ethiopia from March – April 2015. (n =381)

method	species	Sensitivity			Specificity			NPV			PPV		
		%	95% CI		%	95% CI		%	95% CI		%	95% CI	
			lower	upper		lower	upper		lower	upper		lower	upper
Mini Parasep® SF	<i>A. lumbricoides</i>	92.7	0.878221	0.958046	100	0.97517	1	93.1	0.884624	0.960343	100	0.973669	1
	Hookworm	42.7	0.299659	0.567265	100	0.985438	1	91.0	0.874612	0.936973	100	0.828285	1
	<i>S. mansoni</i>	93	0.868906	0.965839	100	0.98121	1	94.9	0.93309	0.983014	100	0.961651	1
	<i>T. trichiura</i>	69.8	0.628994	0.758581	100	0.973376	1	74	0.678036	0.793064	100	0.967395	1
	<i>H. nana</i>	89.6	0.76557	0.96101	100	0.897857	1	98.5	0.96382	0.994541	100	0.897857	1
	<i>E. vermicularis</i>	100	0.309989	1	100	0.987459	1	100	0.987459	1	100	0.309989	1
	<i>Taenia species</i>	96	0.768838	0.997821	100	0.986729	1	99.2	0.982061	0.999854	100	0.821902	1
	Any species	92.7	0.863794	0.931333	100	0.915811	1	72.7	0.511381	0.754399	100	0.98403	1
Kato-Katz	<i>A. lumbricoides</i>	62	0.546774	0.687934	100	0.97517	1	72.1	0.662196	0.773931	100	0.961027	1
	Hookworm	12.5	0.055902	0.24686	100	0.985438	1	86.9	0.829537	0.900639	100	0.560935	1
	<i>S. mansoni</i>	44.6	0.359806	0.53573	100	0.98121	1	77.7	0.726955	0.820491	100	0.922613	1
	<i>T. trichiura</i>	82.4	0.763809	0.872488	100	0.973376	1	83	0.771294	0.876791	100	0.972298	1
	<i>H. nana</i>	19.1	0.094378	0.331044	100	0.985784	1	89.8	0.858387	0.923513	100	0.628811	1
	<i>E. vermicularis</i>	66.7	0.125335	0.982347	100	0.987459	1	99.7	0.983044	0.999862	100	0.197868	1
	<i>Taenia species</i>	29.2	0.134383	0.512475	100	0.986729	1	95.5	0.926813	0.972466	100	0.560935	1
	Any species	80	0.750422	0.839981	100	0.915811	1	64	0.460861	0.644808	100	0.981988	1
McMaster technique	<i>A. lumbricoides</i>	58.3	0.510019	0.653257	100	0.97517	1	70.3	0.643507	0.7558	100	0.958673	1
	Hookworm	58.9	0.450057	0.716284	100	0.985438	1	93.4	0.901108	0.956723	100	0.870171	1
	<i>T. trichiura</i>	30.2	0.241419	0.371006	100	0.973376	1	55.2	0.495291	0.606891	100	0.927311	1
	<i>H. nana</i>	70.8	0.557404	0.825999	100	0.985784	1	96.0	0.931665	0.976901	100	0.873597	1
	Any species	55.2	0.496196	0.606229	100	0.915811	1	57.6	0.206409	0.332806	100	0.974096	1

CI- confidence interval, PPV-positive predictive value, NPV- negative predictive value

Agreement of the test results

The agreement of Mini Parasep® SF faecal parasite concentrator technique with the ‘standard’(combined results of the three methods) was perfect in detecting *A. lumbricoides*, *H. nana* and *S. mansoni* infections, substantial in detecting *T. trichiura* and moderate in detecting Hookworm infections(Table-5). The Kato Katz method agreed perfectly in detecting *T. trichiura*, substantially in diagnosing *A. lumbricoides* and moderately in diagnosing *S. mansoni* infections with combined three techniques (Table-5). However, the agreement of the Kato Katz and the combined three techniques was slight in the case of Hookworm and *H. nana*infections (Table-5).

Table- 5. Agreement of the Mini parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster egg counting techniques with the ‘standard’ (combined results of the three methods) in diagnosing intestinal parasite infection among school children in Wosha Soyama primary school, from March – April 2015. (n =381)

Methods	Intestinal parasites	Kappa-value
Mini Parasep® SF faecal parasite concentrator	<i>A. lumbricoides</i>	0.93
	Hookworm	0.56
	<i>S. mansoni</i>	0.87
	<i>T. trichiura</i>	0.68
	<i>H. nana</i>	0.94
Kato Katz thick smear	<i>A. lumbricoides</i>	0.62
	Hookworm	0.20
	<i>S. mansoni</i>	0.51
	<i>T. trichiura</i>	0.81
	<i>H. nana</i>	0.29
McMaster egg counting technique	<i>A. lumbricoides</i>	0.58
	Hookworm	0.71
	<i>S. mansoni</i>	NA*
	<i>T. trichiura</i>	0.29
	<i>H. nana</i>	0.81

NA*= No parasite found or not detected

The McMaster egg counting technique and the ‘gold standard’ agreed perfectly in detecting *H. nana*, substantially in detecting Hookworm infection and moderately in detecting *A. lumbricoides* but the agreement in the case of *T. trichiura* was poor (Table-5).

4.2. Discussion

In this study, the prevalence of any parasitic infection was 86.1% (328/381). *A. lumbricoides*, *T. trichiura*, *S. mansoni*, *H. nana*, Hookworms, *E. vermicularis*, *E. histolytica /dispar*, *G. lamblia* and *Taenia* species were the species detected. *T. trichiura* was the leading helminths detected followed by *A. lumbricoides* and *S. mansoni*. The least prevalent intestinal parasite detected was *E. vermicularis*. The low prevalence of this parasite in our study could be due to the Stool is not an appropriate specimen (For this worm a sticky tape sample from the anus is required). This finding agrees with the cross-sectional study done in 2007 in Shesha Kekele, around Wondo Genet town where a high prevalence (85.1%) of intestinal parasites infection among children with the three more prevalent intestinal parasites (*T. trichiura*, *S. mansoni* and *A. lumbricoides*) were detected (Nyantekyiet *al.*, 2010). This agrees with current study even though it was done a few years back. Moreover, although an ongoing effective de-worming project through the distribution of albendazole, ivermectin and praziquantel at a primary health care approach has been carried out in the study area, the prevalence, as shown in our data, has not decreased since then.

Comparison of the diagnostic performance of these three methods with the combined results showed as Mini Parasep® SF faecal parasite concentrator was the simplest, fastest method to perform, enclosed system, require less equipment's and detected many species which were detected in the study area. It also showed less egg count when the mean egg counts compared to Kato Katz thick smear and McMaster techniques for each parasite, except it showed higher mean egg count for *H. nana* and Hookworm than Kato Katz thick smear. This is due to both Kato Katz thick smear and McMaster techniques have higher multiplication factor than it (WHO, 2012; Zajac & Conboy, 2012).

Kato-Katz thick smear was not simple to perform and requires different diagnosing time for each parasite. By this method Hookworm egg disappears (disintegrated) quickly and not observed after one hour because it clear rapidly and will no longer be visible after 30-60 min (WHO,

2012). The possible reasons for poor performance of the Kato-Katz in detecting Hookworm infection could be explained by the following facts. First, Hookworm has lower egg laying capacity, more likely to be missed by Kato-Katz. Second, Hookworm eggs disappear due to glycerin (which destroys Hookworm eggs over time) when long time delays occur between Kato-Katz smear preparation and microscopic examination as previously reported (Dacombe *et al.*, 2007). It is also reported as it may be related to rapid degeneration of delicate Hookworm eggs with time (Tarafder *et al.*, 2010). Furthermore, unlike Mini parasep ® SF faecal parasite concentrator, and McMaster methods small amount of stool samples was processed in Kato-Katz technique. This agrees with the previous report as the chance of detecting Hookworm infection by Kato-Katz from small amount of faecal materials suggested to be lower (Lin *et al.*, 2008). Therefore, small amount of faecal material used in Kato Katz technique may be the reason for lower detection capacity of Kato - Katz.

H. nana eggs also not seen when examined after one hour by Kato-Katz. It showed low detection capacity for *H. nana* compared to both Mini parasep ® SF faecal parasite concentrator and McMaster egg counting techniques. So Kato-Katz is not the best technique to assess the prevalence and intensity of infection of *H. nana*. One possible explanation could be that *H. nana* eggs are very small (30–40µm) and yellowish/transparent and thin-shelled eggs and disappear during the clearing process in a short time of 30-120 minutes (Knopp *et al.*, 2006). But, *A. lumbricoides*, *T. trichiura*, *S. mansoni*, and *Taenia* species were better detected after one hour of Kato-Katz thick smear preparation. From the nine species detected cyst of *E. histolytica* and cyst of *G. lamblia* were not detected by this method. This agrees with previously report as Kato - Katz was unsuitable for detection of cysts (Knopp *et al.*, 2006).

McMaster egg counting technique is time consuming method to perform next to Kato-Katz and also showed lesser performance than Mini Parasep® SF faecal parasite concentrator and Kato Katz thick smear. It detects only four species from the nine species detected by the combined results of the three methods as gold standard methods. But it showed better performance for diagnosis of Hookworm eggs than Mini Parasep® SF faecal parasite concentrator and Kato-Katz thick smear (8.7%, Vs 6.3% and 1.8%), respectively and better performances for *H. nana* than Kato Katz thick smear (8.9% Vs 2.4%). It also showed higher mean egg count for *H.nana* and Hookworm than the two methods.

Comparison of prevalence, sensitivity, specificity, positive and negative predictive values and intensity of intestinal parasitic infection of the three methods showed: as more prevalence of intestinal parasites were detected by Mini Parasep® SF faecal parasite concentrator followed by Kato-Katz thick smear and McMaster methods (77.7%, 68.8% and 47.5%), respectively. Mini Parasep® SF faecal parasite concentrator showed more sensitivity and negative predictive value of (90.2% & 72.7 %) than Kato-Katz thick smear (80.0% & 64 %) and McMaster techniques (55.2% & 57.6%). These three methods also showed different sensitivity for each detected parasites. The sensitivity of Mini Parasep® SF faecal parasite concentrator detected in this study on preserved samples almost agrees with the sensitivity obtained when it compared with modified formol-ether on fresh samples, which was 90.2% vs 93% respectively (Ikeh & Elujola, 2015). In general Mini Parasep® SF faecal parasite concentrator and Kato Katz thick smear have the ability to detect more helminths egg. Mini Parasep® SF faecal parasite concentrator in addition detected cysts of protozoan parasites. McMaster technique detected only four species from the nine detected species. It didn't detected *S. mansoni* egg which was the 3rd more prevalent helminths detected in this study. This may be due to the flotation solution concentrated sodium chloride (NaCl) which has no ability to float this parasite by McMaster technique (Zajac and Conboy, 2012). This agrees with the previous report as a particular advantage of the Kato-Katz method than McMaster methods for its ability to simultaneously detect STH and *Schistosoma* species at low cost (Nikolay *et al.*, 2014) which currently shown by this study in addition as it is also true for Mini Parasep® SF faecal parasite concentrator for its ability to simultaneously detect STH and *Schistosoma* species.

Concerning the intensity of infection from the helminths for which the Value for calculation was available in WHO criteria, *S. mansoni* showed more heavy infection in the study area followed by *A. lumbricoides* and *T. trichiura*. Hookworm showed only light infection. Kato-Katz thick smear showed better performance in assessing intensity of infection for *A. lumbricoides*, *T. trichiura*, *S. mansoni*, *E. vermicularis* and *Taenia species* than the two methods. This agrees with pervious study reported from north Argentina except for Hookworm as Kato-Katz thick smear has higher eggs count per gram of stool (EPG) for Hookworm than McMaster (Barda *et al.*, 2014). McMaster also showed better performance for *H. nana* and Hookworm mean egg count than the two methods. Mini Parasep® SF faecal parasite concentrator also showed higher mean egg count for *H. nana* and Hookworm than Kato-Katz thick smear. In general single slide Kato-

Katz thick smear showed better performance for more helminths egg count in EPG stool from the three methods compared. This may be due to the Preservation (10% formaldehyde) is known to alter the morphology/density of eggs, resulting in false negative test results and an underestimation of EPG stool (Foreyt 1986) which was true both for Mini Parasep® SF faecal parasite concentrator and McMaster methods.

Mini Parasep® SF faecal parasite concentrator showed more kappa value /agreement with the combined value, for more detected parasites than the two methods. Kato - Katz thick smear showed better agreement with the combined results than the two only for *T. trichiura*. McMaster also showed more agreement for only Hookworm than the two methods.

Overall, the most time-consuming method to be processed was Kato-Katz (mean time 48 min/sample).The second time consuming next to Kato-Katz was McMaster (7 min/sample) as also previously reported by Barda *et al.*, 2014. Mini Parasep® SF faecal parasite concentrator was the quickest to process (mean time 3 min/sample).The waiting time for the clarification of the eggs for Kato-Katz and for the floatation of the eggs for the McMaster were the factors that accounted most significantly for the length of processing single samples which agrees with previously report (Barda *et al.*, 2014) . It must be underlined, however, that for both techniques, when processing multiple samples, the time (min/slide) decreased significantly. Reading time was faster for the McMaster and Mini Parasep® SF faecal parasite concentrator as they were clear slides, whilst the Kato-Katz were slides more difficult to read due to the presence of debris and artifacts.

In addition, McMaster method and Miniparasep ® SF faecal parasite concentrator have several advantages when a large number of samples need to be examined because the microscopy is readily performed, and all parasites can be examined simultaneously, in contrast to the Kato-Katz method where different clearing times for the different STH require re-examination at times optimal for different species (*Speich et al.*, 2010). Another advantages of Mini parasep ® SF faecal parasite concentrator and McMaster methods than Kato Katz, was that both can be performed on fixed stools, enabling processing at a later date in a central laboratory. This can help to increase the quality control process and overcomes some of the logistical difficulties in

examining fresh stool samples in the field on the day of collection even though it has its own side effects on the morphology and detection of intensity of infection.

Mini parasep ® SF faecal parasite concentrator has health and safety benefits that includes: totally it is enclosed/sealed process, reduced reagent volumes, no cleaning required, single use, no sample contamination, and ready to use systems as also previously reported (Moody *et al.*, 2013). In addition it has performance benefits includes:-optimum sample recovery, enhanced sample clarity, rapid four step process, human resources optimized and easy patient identification (Moody *et al.*,2013). But both Kato Katz and McMaster need cleaning of materials used and there also a great chance of contamination.

4.3. Limitation of the study

- Lack of a gold standard for perfect comparison purpose of the methods
- Lack of guideline to classify the intensity of infection for *E. vermicularis*, *H. nana*, and *Taenia* species

5.0. Conclusion and Recommendation

Mini parasep ® SF faecal parasite concentrator was simple, fastest and contamination free and detected many helminths including cysts of protozoan parasites than the two methods. It had low egg counting ability compared to the two methods, except higher mean egg count for *H. nana* and Hookworm than Kato -Katz thick smear. Kato- Katz also showed better performance than McMaster except for Hookworm and *H. nana*. It was also more time consuming than both methods. McMaster showed better performance for Hookworm than both methods. In addition it had better performance than Kato- Katz for *H. nana*. In general Mini parasep ® SF faecal parasite concentrator had more sensitivity (90.2%) and negative predictive value (72.7%) than both methods. Kato-Katz thick smear also had more sensitivity (80% vs 55.2%) and negative predictive value (64% vs 57.6%)than McMaster egg counting technique. Moreover, Kato Katz thick smear showed better performance in assessing intensity of infection for most helminths detected than the two methods. Intestinal parasitic infections showed alarming prevalence {86.1% (328/381)} and intensity of infection in study area. The three most prevalent helminths detected in this area were *T.trichiura* 53.8%, *A.lumbericoides* 50.7% and *S.mansoni* 34.1% respectively. As the prevalence and intensity of parasitic infection in this area was high it is indicated to strength health promoting activities.

For Mapping priority areas for control, monitoring and evaluation of programs impact, or for surveillance purpose as well as for the routine diagnosis of intestinal parasites it is better to use Mini parasep ® SF faecal parasite concentrator than Kato-Katz thick smear and McMaster egg counting technique. Evaluation of Mini parasep ®SF faecal parasite concentrator with other intestinal parasitic diagnostic methods also is recommended.

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ANNEXES

ANNEX – I: - Data Collection Instrument

LABORATORY INVESTIGATION

a. Stool Examination Result

1. Macroscopic

Sample No /code	Age	Sex	Class/grade	formed	watery	mucoïd	bloody	soft
1								
2								
3								
.								
381								
total								

2. Microscopic

Sample No	Age	Sex	Class/grade	Methods		
				Mini Parasep® SF Faecal Parasite	Kato Katz thick smear	McMaster
1						
2						
3						
.						
381						
Total						

Date _____

Signature _____

Annex II. Materials and reagents required

A. Mini Parasep® SF Faecal Parasite Concentrator

1. Microscope
2. Microscope slide
3. Normal Saline (0.85%)
4. Glove
5. Centrifuge with 15ml centrifuge buckets
6. Mini parasep ®SF apparatus prefilled with 3.3ml 10% formalin and one drop of Triton X-100
7. Micro-pipette of 1000 ul
8. Green tip
9. Biohazard bug
10. D'Antoni's iodine or Gram's iodine
11. Applicator stick
12. Stool cup

B. Kato Katz

1. Applicator sticks, wooden.
2. Screen, stainless steel, nylon or plastic 60-105 mesh.
3. Template, stainless steel, plastic, or card board. Templates of different sizes have been produced in different countries. A hole of 9 mm on a 1 mm thick template will deliver 50 mg of faeces; a hole of 6 mm on a 1.5 mm thick template, 41.7 mg; and a hole of 6.5 mm on a 0.5 mm thick template, 20 mg. The templates should be standardized in the country and the same size of templates should always be used to ensure repeatability and comparability of prevalence and intensity data.
4. Spatula, plastic.
5. Microscope slides (75 x 25 mm).
6. Hydrophilic cellophane, 40-50 mm thick, strips 25 x 30 or 25 x 35 mm in size.
7. Flat-bottom jar with lid.
8. Forceps

9. Toilet paper or absorbent tissue

10. Newspaper.

11. Glycerol-malachite green or glycerol-methylene blue solution (1 ml of 3% aqueous malachite green or 3% methylene blue is added to 100 ml of glycerol and 100 ml of distilled water and mixed well). This solution is poured onto the cellophane strips in a jar and left for at least 24 h prior to use.

C. for McMaster

1. Two beakers or plastic containers

2. Balance

3. Tea strainer, cheesecloth or dental napkin

4. Measuring cylinder

5. Stirring device (fork, spatula, tongue depressor)

6. Pasteur pipettes and rubber teats

7. Flotation fluid (choice of solution dependent on species expected to be present and availability of reagents)

8. McMaster egg counting chamber

9. Compound microscope

10. Preservatives (10% formalin)if delay for more than 1hour is necessary

11. Stool cup

12. Applicator stick

13. Gauze bandage or toilet paper

D. Others required materials

1. Pencil

2. Marker, Pen ,ruler

3. Floppy disk

4. Typing and duplicating paper

5. Flash disk

6. 70% Alcohol

Annex III: Manufacturer instruction and Standard Operating Procedures (SOPs)

A. Mini Parasep® SF Faecal Parasite Concentrator(according manufacturer instruction APA057-V3 07/2014, www.apacor.com or e-mail on: sales@apacor.com)

Procedure

See label for storage conditions and expiry date

Please adhere to the following guidelines when handling Parasep. To avoid cross contamination the Parasep device should remain closed at all times except when introducing the sample or when retrieving the final concentrated sample for examination.

1. Sample Preparation

Fresh Samples

Unscrew lid and add 3.3ml of **10% formalin** and one drop of **Triton X-100** to the mixing tube. Introduce a level scoop (pea size/0.4gm) of faecal sample **OR** Mini parasep® Pre-filled with 3.3 ml 10% Formalin fixative & one drop Triton X-100. Use the spoon at the end of the Mini Parasep filter.

Preserved Samples

Shake or vortex the incoming preserved sample to thoroughly mix. Transfer 0.3ml = 0.3g of sample into the Mini Parasep® SF mixing chamber. Add 3.0ml of 10% Formalin/water plus 2 drops of Triton X.

2. Emulsification

Seal Mini Parasep by screwing in the filter thimble and conical tube.

Vortex or shake to emulsify with the sedimentation cone pointing upwards.

The faecal sample may be left for 24hr to soften and ensure complete bactericidal action has taken place.

Note: To calculate the required RPM for any centrifuge.

$$\text{RPM} = \sqrt{g/1.12r} * 1000$$

RPM - rotor speed in revs/min.

g - Centrifugal force (max.1000g)

r - Radius, horizontal distance between sedimentation cone tip and spindle center measured in mm.

3. Centrifugation

Insert Mini Parasep and centrifuge at 500g for two minutes.

Mini Parasep fits all 15ml centrifuge buckets.

4. Examination

Direct Method

Unscrew and discard the filter thimble and mixing tube. Pour off all the liquid above the sediment.

Pipette one drop of **saline** onto a slide, add one drop of deposit to the saline. Mix sample and cover with cover-slip. The deposit will be examined microscopically using physiological saline and D'Antoni's iodine for the eggs, trophozoites and larvae of intestinal parasites.

OR

FE Workstation Method

Unscrew and discard the filter thimble and mixing tube. Pour off all the liquid above the sediment.

Add 1ml **water or 1ml of Daisy's FE Stain** to the sediment. Shake or vortex to re-suspend sample.

Insert Aspirator into suspension and press SAMPLE to draw 80µl into the Optical Slide. (Refer to FE instruction manual). EPG was reported by multiplying the amount of eggs counted by 2 (Levecke et al., 2009)

B. Kato Katz thick smear method (WHO, 2012)

1. Place a small amount of faecal material on newspaper or scrap paper and press the small screen on top so that some of the faeces are sieved through the screen and accumulate on top.
2. Scrape the flat-sided spatula across the upper surface of the screen to collect the sieved faeces.
3. Place template with hole on the center of a microscope slide and add faeces from the spatula so that the hole is completely filled. Using the side of the spatula pass over the template to remove excess faeces from the edge of the hole (the spatula and screen may be discarded or, if carefully washed, may be reused).
4. Remove the template carefully so that the cylinder of faeces is left on the slide.
5. Cover the faecal material with the pre-soaked cellophane strip. The strip must be very wet if the faeces are dry and less so if the faeces are soft (if excess glycerol solution is present on upper

surface of cellophane wipe with toilet paper). In dry climates excess glycerol will retard but not prevent drying.

6. Invert the microscope slide and firmly press the faecal sample against the hydrophilic cellophane strip on another microscope slide or on a smooth hard surface such as a piece of tile or a flat stone. The faecal material will be spread evenly between the microscope slide and the cellophane strip. It should be possible to read newspaper print through the smear after clarification.

7. Carefully remove slide by gently sliding it sideways to avoid separating the cellophane strip or lifting it off. Place the slide on the bench with the cellophane upwards. Water evaporates while glycerol clears the faeces.

8. For all except Hookworm eggs, keep slide for one or more hours at ambient temperature to clear the faecal material prior to examination under the microscope. To speed up clearing and examination, the slide can be placed in a 40°C incubator or kept in direct sunlight for several minutes.

9. *Ascaris* and *Trichuris* eggs will remain visible and recognizable for many months in these preparations. Hookworm eggs clear rapidly and will no longer be visible after 30-60 minutes. *Schistosom* eggs may be recognizable for up to several months but it is preferable in a schistosomiasis endemic area to examine the slide preparations within 24 hours.

10. The smear should be examined in a systematic manner and the number of eggs of each species reported. Later multiply by the appropriate number to give the number of eggs per gram of faeces (by 20 if using a 50 mg template; by 50 for a 20 mg template; and by 24 for a 41.7 mg template).

C. McMaster egg counting technique (according to Zajac and Conboy, 2012)

Procedure:

1. using a tongue depressor, weigh out 2 to 4 gm of feces into beaker.
2. Break up the faecal pellets and add the correct amount of flotation solution to the feces to make a slurry. You'll need a total of 56 ml flotation solution for 4 g of feces, 42 ml for 3 g or 28 ml for 2 gm. It is easiest to add just a little of your floatation solution to first break up the faecal pellets and then add the remainder of the solution. For example, for 4 g of feces, you can add about 20 ml flotation solution to help break up the feces using the tongue depressor to break

lumps. Then bring the slurry up to the 60 ml mark on your beaker using the remainder of your flotation solution.

3. Pour the solution through a tea strainer into a clean cup.

4. After letting the solution strain for a few minutes, tap the strainer against the cup until you just have a ball of feces left in the cup

5. Discard feces

6. Add a stir bar, and stir on a magnetic stirrer at medium speed for 5 min. **OR** put in a leak-proof jar and shake vigorously for 5 minutes.

7. at the end of 5 minutes, while mixture is still stirring, draw about 1 ml faecal suspension from the upper layers of the slurry into your syringe.

8. Load one side of counting chamber carefully to avoid producing bubbles – each chamber holds about 0.15 ml of slurry and repeat sampling and loading procedure for second side of chamber.

9. Let preparation stand a minimum of 5 min (examine it at least by 20 min.) If allowed to sit too long, the eggs will fall away from the gridlines.

10. Place chamber on microscope and examine with 10 X objective (Adjust the focus until you can see grid lines clearly and then refine your focus to the air bubble layer).

11. Count eggs in both sides of chamber- each chamber or grid has six sections. Do not count eggs outside the grid. Calculate the number of eggs per gram of feces: (side 1 + side 2) X 50

Preparation of Flotation solutions:

Saturated Sodium Chloride:

Table salt 1 pound box

Tap water 3 quarts

Heat in pan with stirring until boiling, then let cool at room temp. The solution will look cloudy and some material will precipitate - this is OK. Pour clear part of solution into a dispensing container of some kind.

Store at room temperature. Do not refrigerate as additional solute will precipitate.

Note: Faecal floatation solutions are also commercially available, but are significantly more expensive than using this recipe (although not high dollar).

Clean up of Materials:

- Rinse all materials used for the slide preparation with tap water between each sample. If reusing the dropper or syringe, rinse with flotation solution after rinsing with tap water.
- You can apply acetone to faecal cups to erase sharpie marker
- **DO NOT** apply acetone near McMaster slides as this will cause them to become cloudy and unusable!
- Before putting materials away for an extended period of time, wash them with warm, soapy water, rinse with tap water, and then rinse with deionized water.

Annex IV. Information sheet and Oral consent form

Information sheet

Title: Comparison of diagnostic performance of Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster methods for the diagnosis of intestinal parasitic infections among Wosha Soyama primary school students in Wondo Genet, southern Ethiopia from March - April 2015

You are being invited to take part in a research project will be carried on Comparison of diagnostic performance of three diagnostic methods: Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster methods for the diagnosis of intestinal parasitic infections. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to hear the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Intestinal parasites are among the most common cause of human infections which are distributed throughout the world and cause thousands of avoidable outpatient morbidity and mortality, especially in school-age children. They are also the leading cause of gastrointestinal pain, malnutrition, malabsorption, anaemia, mental retardation and other diseases. The diagnosis of these infections commonly relies on the detection of parasites egg, larvae, cyst, oocyst, or trophozoite in stool. However, infections often escape diagnosis due to inadequate skill, inappropriate diagnostic method efficacy, inappropriate sample collection technique and rule out absence of parasite by examining only single specimen and examining this single specimen by only direct wet mount which is less sensitive and less specific. So, performance evaluation of diagnostic tests is critical in the search for better diagnostic approaches. In this study it is proposed to compare and evaluate the performance of Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster methods for the diagnosis of intestinal parasitic infections in primary school children in Wosha Soyama primary school students in Wondo Genet, southern Ethiopia from March - April 2015

So, you are chosen to participate in this study by systematic random Sampling technique and other 380 students will be selected for this study by this method from your school. It is up to you

to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and you can still withdraw at any time without it affecting any benefits that you are entitled to in any way. It is not expected from you to give a reason to stop.

If you are willing to participate and didn't treated before in the past four months for these intestinal parasites, you will be requested to give single fresh stool samples about 5 gm at the time of visit for intestinal parasite diagnosis by three methods will be compared. By doing this no any reasonably foreseeable discomforts, disadvantages and risks will happen on you. Being participating on this research has many advantages for you. Out of these you can know your health situation concerning these intestinal parasites and get appropriate treatment free without seeking for treatment in health institution after you are ill and get more complication by being within your school without being absent from class.

All the information that we collect about you during the course of the research will be kept strictly confidential. You will not be able to be identified in any reports or publications.

This project is funded by WHO through ALIPB for the kits needed for this study and from Addis Ababa University concerning the per diem and other materials.

This information sheet's copy will be given for each participant. Finally I would like to thank you for taking time to hear the information given and willing to participate.

Contact for further information

Shimeles Adugna Mobile number 0911720233 and email shimeadu39@gmail.com

ስለጥናቱ ኢንፎርሜሽን

የጥናቱ ርዕስ የ Miniparasep ®SF,Kato- Katz እና McMaster የሰገራ ምርመራ ዘዴዎችን አፈጻጸም በኢትዮጵያ ውስጥ በወንድገነት ከተማ ወሻ ሶያማ የመጀመሪያ ደረጃት/ቤት ውስጥ ከመጋቢት እስከሚያዝያ 2007 ዓ.ምማወዳደርነው፡

አንተ በሰገራ ምርመራ አፈጻጸም ማወዳደር ላይ በሦስቱ ዘዴዎች ማለትም "Miniparasep ®SF", በ"Kato- Katz" እናበ"McMaster" በሚደረገው ጥናት እንድትሳተፍ ተመርጠሃል።ከመወሰን በፊትሰምን ጥናቱ እንደሚሰራና ምንን እንደሚያካትት መረዳት አለብህ። ስለዚህ ጊዜ ወስደህ ቀጥሎ የሚሰጡትን መረጃዎች በጥሞና አዳምጠህ ከሌሎች ጋርከፈለክ ተወያይበት። እኛንም ግልፅ ያልሆነ ነገር ካለ ወይንም ተጨማሪ መረጃ ከፈለክ ጠይቀን።ጊዜ ወስድህ በጥናቱ ለመሳተፍ ወይም ላለመሳተፍ ወስን።

የሆድ ውስጥ ትላትሎች በአለም ላይ በጣም የተሰራጩ ና በሚሊዮኖች የሚቆጠሩ ጉዳዮችና ሞትን በተለይ በት/ቤት ባሉ ህፃናቶች ላይ ያስከተላሉ።

እነዚህ የሆድ ውስጥ ትላትሎች ዋናዎቹ የሆድ ህመም፣የምግብ እጥረት፣ምግብ በአግባቡ ለሚፈለገው ሰውነት ክፍል እንዳደርስ ፣የደምማነስ፣ የአይምሮ ዘገምተኛነትና ሌሎች በሽታዎችን ያስከትላል።

የእነዚህ በሽታዎች ምርመራ በብዛት የተወሰነው የትላትሎችን እንቁላል፣ እጭ፣ ቀፎ አቸውን እናትሮፎዘይት በሰገራ ውስት በመመለከት ነው ። ይሁን እንጂ እነዚህ በሽታዎች ምርመራ ከእውቀት ማነስ የተነሳ፣ አመቺ ባልሆነ የምርመራ ዘዴ ብቃት፣ አስፈላጊ ባልሆነየ ናሙና አወሳሰድ ዘዴ እና አንድ ጊዜ ብቻና ሙናውን በመውሰድ በቀትታ ናሙናውን በእርጥቡ ወዲያውኑ በማይክሮስኮፕ በመመርመር ከምርመራ ያመልጣሉ። ስለዚህ የምርመራ ዘዴዎች ብቃታቸውን መመርመር የተሻለ ዘዴ ለማግኘት ወሳኝነት አለው። በዚህጥናት የታቀደውየ “Miniprasep ®SF, Kato -Katz” እና “McMaster” የሰገራ ምርመራ ዘዴዎችን በኢትዮጵያው ስጥ በወንድገነት ከተማ በወሻ ሶያማ የመጀመሪያ ደረጃት/ቤት ውስጥ ከመጋቢት እስከ ሚያዝያ 2007 ዓ.ምማወዳደርነው፡

ስለዚህ አንተ በዚህ ጥናት ለመሳተፍ በሎቶሪ ዘዴ ወይንም በቀላሉ አልፎአልፎ በተማሮዎች ብዛትና በሚፈለገው የጥናቱ ተሳታፊዎች ብዛት በሚሰላው ዘዴ የተመረጥክ ሲሆን ሌሎችም 380 ተማሪዎች በዚህ መሰረት የሚመረጡ ይሆናሉ። በጥናቱ መሳተፍህ ወይም አለመሳተፍህ ባንተ ፍቃድ የሚወሰን ነው።

በጥናቱ ለመሳተፍ ከወሰንክ ይህ የመረጃ መስጫ ጽሁፍ የሚሰጥህና ከተሰጠህም በኋላ በማንኛውም ጊዜ ማቋረጥ ከምታገኘው ማንኛውም ጥቅማጥቅም በማንኛውም መንገድ የማይገድብህ ነው። ምንም ምክንያት ጥናቱን ለማቋረጥ ማቅረብ አይጠበቅብህም። ነገርግን ለመሳተፍ ፍቃደኛ ከሆንክክ 4 ወራት ወዲህ ለእነዚህ የሆድ ውስጥ ትላትሎች የተሰጠ መድሀኒት ከሌለህ እስከ አምስትግራም ትኩስ ሰገራ በጥናቱ ጊዜ አንዴ በሦስቱም ዘዴዎች ለመርመር እንድትሰጥ ትጠየቃለህ። ይህን በማድረግህ ምንም አይነት ጉዳት የሚደርስብህ የለም። በጥናቱ መሳተፍ ብዙ ጥቅማትቅሞች አሉት። ከነዚህም የሆድ ትላትሎችን በተመለከተ ያለህበትን የጤንነት ሁኔታ የምታውቅበትና በሰገራህ ውስጥ ትላትሎቹ ከተገኙ በነፃ መድሀኒት የማግኘትና ከመታመምና ከተለያዩ ውስብስብ የጤና ችግሮች ከመከሰታቸውና ለህክምና የትምህርት ጊዜን ና ገንዘብን ከማውጣት የሚያተርፍ ነው። ሁሉም ስለጤንነትህ የሚጠቅሱ የምርመራ ውጤቶች ሚስጢራዊነታቸው በሚገባ የሚጠበቅ ነው። አንተ በማንኛውም ሪፖርት ወይም ህትመት አትታወቅም።

ይህ ጥናት የሚካሄደው የአለም የጤናድርጅቶች በአክሊሎሎማ የፓቶባዮሎጂ ኢኒስቱቱ ዩት በኩል በሚሰጠው የምርመራ መሳሪያዎችና ከአዲስ አበባ ዩንቨርሲቲ በተሰጠው የአበልና የተለያዩ ቋሚ መሳሪያዎች ነው።

ይህ የመረጃ ጽሁፍ ኮፒ ው ለጥናቱ ተሳታፊ ይሰጣል በመጨረሻም ጊዜህን መስዎት አድርገህ በቃል የተሰጠውን መረጃ በማዳመጥና ለመሳተፍ ፍቃደኛ በመሆንህ አመሰግናለሁ።

ለተጨማሪ መረጃ

ሽመልስ አዳኛ ሞባይል ቁጥር 09 11 72 02 33 Email:- shimeadu39@gmail.com መጠቀም ትችላላችሁ።

ማሳሰቢያ:- ይህ መረጃ ለውንድ ጾታ የተዘጋጀ ሲሆን ለሌትም መጠቀም ይቻላል።

Written consent form

Code No _____

Name of the study participant (optional) _____

Age_____ Sex_____, grade_____

Name of investigator _____ Study site/school_____

I have been informed about a study that plans to evaluate the diagnostic performance of Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster methods for the diagnosis of intestinal parasitic infections among school children in Wondo Genet Wosha Soyama Primary School students in Ethiopia from March –April 2015 which helps in understanding the prevalence and intensity of parasitic infection in the study areas and evaluate the diagnostic performance of the above three methods. At the same time, it enables concerned body in designing better control and preventive measures of parasitic diseases in the study area.

For this study, I was requested to give stool sample for intestinal parasites identification. I was informed that I will get proper therapy if I found to be positive for any intestinal parasites. The investigator has also briefed me that there would be no health related risks associated with the sampling procedure. He also informed me that all laboratory results would be kept in secret. Moreover, I was clearly informed that my participation in this study is completely voluntary and I have right to withdraw from participating in this study and in so doing there will be no impact on the overall management of my conditions. Refusal to participate will not result in loss of medical care provided or any other benefits. I was given enough time to think over before giving my oral informed consent. It is therefore; with full understanding of the situation that I gave informed consent and cooperate at my willing in the course of the study.

Name (Wittiness) 1. _____ Signature _____ Date _____

2. _____ Signature _____ Date _____

3. _____ Signature _____ Date _____

Name of guardian/ parent's _____ Signature _____ Date _____

Name (Investigator) _____ Signature _____ Date _____

የጹሁፍ ስምምነት የሚሞላበት ፎርም

መ.ቁ _____

የጥናቱ ተሳታፊ ሰው ስም _____ እድሜ _____ ጾታ _____ ክፍል _____

ጥናቱን የሚያካሄደው ሰው ስም _____ የጥናቱ ቦታ _____ የት/ቤቱ ስም _____

እኔ የ” Miniprasep ©SF faecal parasite concentrator, Kato- Katz thick smear” እና” McMaster” የሰገራ ምርመራ በሆድ ውስጥ ሰለሚገኙ ትላትሎች ስርጭትና ጫና ላይ በኢትዮጵያ ውስጥ በወንድገነት ከተማ ወሻ ሶያማ የመጀመሪያ ደረጃት/ቤቶት ውስጥ ከመጋቢት እስከሚያዝያ 2007 ዓ.ም ስለሚካሄደው ጥናት አላማ ሙሉ ማስረጃ ተሰጥቶኛል።

ለዚህ ጥናት እኔም ሰገራ በሆድ ውስጥ ሰለሚገኙ ትላትሎች ምርመራ እንድሰጥ ተጠይቄያለሁ። እነዚህትላ ትሎች እኔ በምሰጠው ናሙናው ስጥየሚገኙ ከሆነ አስፈላጊው ህክምና የሚሰጠኝ እንደሆነም ተነግሮኛል። ጥናቱን የሚያካሄደው ሰው ናሙናውን በመስጠት ምንም አይነት ጉዳት የማይመጣብኝ መሆኑን ነግሮኛል። የምርመራው ውጤትም በሚስጥር እንደሚጠበቅም ነግሮኛል።

ከሁሉም በላይ በዚህ ጥናት መሳተፍ በፍቃደኝነት ላይ የተመሠረተ መሆኑና በኔ ላይ የሚደርስ ጉዳት እንደ ሌለ ተነግሮኛል። በጥናቱ ለመሳተፍ ፍቃደኛ አለመሆን ለሚደረገው የጤና እንክብካቤና ሌሎች ጥቅማጥቅሞችን የማያሳጣኝ መሆኑ ተነግሮኛል። ለመሳተፍ ፍቃደኛ ከመሆኔ በፊት እንዳሰብበት በቂ ጊዜ ተሰጥቶኛል።

ስለዚህ በጥናቱ ለመሳተፍ የወሰንኩት ስለሁኔታው በሚገባ ከተረዳሁ በኋላ በጥናቱ ሂደት ውስጥ በፍቃደኝነት ለመሳተፍ ተስማምቻለሁ።

የምስክሮች ስም

1ኛ..... ፊርማ..... ቀን.....

2ኛ..... ፊርማ..... ቀን.....

3ኛ..... ፊርማ..... ቀን.....

የአሳዳጊ የቤተሰብ ስም..... ፊርማ..... ቀን.....

ANNEX V: DECLARATION

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

Name of the candidate Shimeles Adugna

Signature _____

Place Addis Ababa University

Date of submission 06/ 09/ 2015

This thesis will be submitted for examination with our approval as university advisors.

Name of Advisor 1. Tadesse Kebede (Asst.Professor) **Signature** _____

Name of Advisor 2. Berhanu Erko (Professor) **Signature** _____

Name of advisor 3. Zeleke Mokonnen (Assoc Prof.) **signature** _____

Place _____

Date of submission ____/____/____

Name of examiners 1. _____ **Signature** _____

2. _____ **Signature** _____

Place _____

Date of submission ____/____/____