

**Research Article**
**Open Access**

## A Comparison between Quality of Formal Benzene Concentration Technique and other Techniques used for the Detection of Intestinal Parasites in Sudan - Jabal Awliya Locality

Elnour Blkamin Coortkila Dldoom<sup>1</sup>, Elamin Abdalkarim Elamin<sup>1</sup> and Muslih Haroun El Hussien Gamea<sup>2\*</sup>

<sup>1</sup>Department of Medical Parasitology, Al -Neelain University, Sudan

<sup>2</sup>Department of Hematology and Immunoematology, Sudan International University, Sudan

### ABSTRACT

**Introduction:** Intestinal parasites are considered as one of a priority for public health in the world, in the Sudan, they represent a main encumbrance of health helmsmen due to their high risk in the communities causing morbidity and mortality.

**Objective:** The randomness abodes, illiteracy and keep away of using different: concentration techniques, can lead to arduousness management of such infections.

**Method:** 102 randomizes collected stool samples from out patients in public laboratory forms the governmental sector were enrolled in this study. The study subjects were concerned for quality of stool examination techniques slides based on intestinal parasites identification (wet preparation and concentration methods) using some detergents with plausible Performance to investigate the quality and competency of them under microscope.

**Results:** of 102 stool samples contacted as responded, this study showed that, the gasoline was gave positivity more than other chemicals used. Benzene gave congenial results, and enable assimilates to stool concentration techniques solvents for intestinal parasite detection, but it needs further search to be so. And the sensitivity and specificity degree, both are low.

**Conclusion:** The study concluded that accurate diagnosis needs to be making concentration techniques necessary for public and private laboratories services to ensure quality of diagnosis.

### \*Corresponding author

Muslih Haroun El Hussien Gamea, Department of Hematology and Immunoematology, Sudan International University, Sudan.

**Received:** September 02, 2025; **Accepted:** September 12, 2025; **Published:** September 17, 2025

### Introduction and Literature Review

#### Background:

Intestinal parasitic infections are group of the most predominant infections in humans particularly in developing countries. Thusly infections cause chronic fettle in human, which may progress to graver disease. Intestinal parasitic infections are globally endemic and have been described as figuration the greatest single universally cause of illness and disease [1].

Betwixt the parasitic infections, intestinal parasitic infections are most blanketed and they are basks with widely circulating in tropical and sub-tropical regions, especially in sub-Saharan Africa, America, China and East Asia (WHO 2012) [2].

There is need for a proper diagnostic techniques to identify the accessing and new cases in the community. The routinely done stool examination techniques for intestinal parasites are direct wet mount (slain and iodine mount) examination and stool concentration methods such as formal-ether sedimentation and saturated salt flotation method, etc. [3].

The formal-ether concentration technique, which consists of ether as fat solvent for detecting out parasites from intestinal debris and thus increase the positivity rates, is believed to foreground over the other methods. However, this technique has been considered to be still disadvantageous since the use of ether may be hazardous for health to laboratory workers. Ether is explosive, contain anesthetic vapors, has potential cardiovascular depression and narcosis. Moreover, it can be a possible cause of mutagen, if inhaled or absorbed through the skin often with harmful long term health effect like neurotoxicity or cancer [4].

To overcome these disadvantages, several other chemicals have been evaluated as substitute for diethyl ether like ethyl acetate tween, acetone, gasoline [5].

Benzene may considers as fecal concentration technique detergent for concentrate scanty of cyst, eggs, and larvae, in case of more researches as alternative solvent to ether and gasoline. It characterized by disadvantages as that it highly flammable, explosive, volatile. Thus, can cause sensory irritation of upper

respiratory when exposed for 10-20 minutes [6].

This study aimed to evaluating the efficiency of formal-benzene concentration method as newly introduce technique in comparison to the routinely used formal- ether and formal-gasoline concentration techniques for detecting parasites, and to assess the best quality of intestinal parasites materials (cyst, oocyst in protozoa and eggs, larvae in helminthes ) among the three solvents.

### Literature Review

Intestinal parasites are major contributors to the global encumbrance of disease, affecting especially the population living in the developing countries, and are part of the neglected tropical diseases. Generally, soil-transmitted helminthes affect almost 1.5 billion people in the world, cause considerable morbidity and account for an estimated 5.2 million disability adjusted life years [7].

The prevalence of intestinal protozoa infections (especially *G. lambiaintestinalis* and *Entamoeba* spp.) vary in different regions of the world. For example, *Giardia* prevalence is 2–7% in developed countries, whereas it is 20–30% in developing countries [8].

Intestinal parasites are transmitted through many lanes, protozoa for example can transmitted via food and water contamination or mechanically (insects), helminthes are major transmitted via soil (soil-transmitted helminthes) especially the roundworm (*Ascarislumbericoides*), whipworm (*Trichuristrichua*) and the hookworm (*Necator Americans* and *Ancylostoma duodenal*) [9].

The estimated values of 3.5 billion people are affected and 4.5 million are ill as result of intestinal parasite [10].

Dependent on world health organization report on soil-transmitted helminthes, the estimated global encumbrance is more than 1.5 billion people representing 24% of world population, with over 270 million of preschool age children and over 600 million towards school-age children as encumbrance [11].

### Transmission

Transmission of both groups of intestinal parasites (helminthes and protozoa). Helminthes are mainly transmitted from the environment via ingestion of infective stage (fecal-oral transmission) or through skin penetration, protozoa are transmitted via fecal-oral transmission of cyst and oocyst from contaminated food or water or hands [12].

### Biology

Intestinal parasites are differ in their nature whence habitat, reproduce and transmission, and inasmuch other has no intermediate host (*Entamoeba histolytica*), the other basks by one or two intermediate hosts (*Teania* species and *Fascicle hepatica* species respectively) [13].

According to different types of the hosts, intestinal parasites complete their life cycles in the certain host, whereas some need one host for their life cycle (*E.Histolytica*), some need farther host for this purpose (one inter mediate host) as *trichnella spirals*, and the third group complete their life cycle via two intermediate hosts as *Fascicle hepatica* [14].

The environment play an important medium for completion of life cycle of some intestinal parasites (hookworm). Helminthes, often are invades specific host, and reproduce a sexual and sexual and

mature, then release into the environment ready to infect humans, some of them are invades farther creature for making full life cycle to produce infective stage (*schitesoma* species) [15]

Although intestinal protozoa are single-celled eukaryotic organism containing organelles and replicating mainly by binary fission, they need one host for their life cycle to produce infective stage (cyst form) in *Gairdia*.

### Diagnosis of Intestinal Parasites

#### Clinical Diagnosis

The first step as initial for diagnosis of suspected infected patients with intestinal parasites, is assessment clinically a right description of the patient condition's illness through physical examination.

To diagnose intestinal parasites clinically, patient should suffer of many symptoms such abdomen pain, snotty and bloody diarrhea, fever and constipation in protozoan infection in endemic regions or in those were visit these regions within period range between 1-4 weeks as incubation period. In helminthes infected patients, and due to long incubation periods, which range between 6monthes to many years, the symptoms are differ in those in protozoan infections, whereby the symptoms include abdomen pain, vomiting, bloody diarrhea or without blood, weight loss, fatigue, fever and.....etc.

Intestinal parasites treatment based on both clinical and laboratory diagnosis, and it differ from parasite to another, and whereas the parasite belongs to helminthes or protozoan infection, where on the treatment into helminthes varies in both phylum (platy helminthes and Nematode), and thus varies protozoan infection treatment [17].

#### Laboratory based diagnosis

The (WHO) recommended that, to diagnose intestinal parasites, microscope is a classic golden standard machine used for detecting and identification of parasites (cyst, eggs, larvae and oocyst). This is consider as last step in the all techniques, and preceded by different procedures [18].

#### Microscopic Examination

The search for eggs and larvae of helminthes (and of ciliates) is classically done using the 10x objective. The entire preparation is examined. To accomplish this, oneshould work systematically. Always start at a corner of the cover slip and work in a straight line from the chosen corner towards the opposite side. Once there, move one row aside and work back until the entire preparation has been examined. Always proceed by looking at the next microscopic field with a small overlap: when a field has been examined, an object in this field is chosen, and is brought towards the opposite side of the field. This second field is then examined. When parasitic structures are found, details are examined at 40x objective [19].

#### Wet Preparation Techniques

Saline, iodine wet mount preparation; the both solutions are used mainly in this procedure to detected motile stages (trophozoites and larvae), red blood cells leukocytes in saline preparation and protozoan cyst in lugal,s iodine, and the stool must be fresh.

Heine negative staining; This technique considered as temporary preparation and useful rapid screening for cryptosporidium species, in doubtful or equivocally stools sample, confirmation must be done by using modified Ziel-Neelsen staining.

#### Concentration (Sedimentation and Flootation) Techniques

These procedures allow for the detection of parasitic elements

(eggs, larvae, oocysts and cysts) that may be missed when examining only a direct wet smear [19].

The floatation technique allows separation of parasitic elements from the coarsest organic debris, using a high specific density floatation solution. Eggs, cysts and oocysts, with a specific density lower than the floatation solution, will rise to the top of the suspension. The specimen can be fresh or fixed stool. The most widely used floatation solutions are zinc sulfate solution and sodium chloride. Heavy eggs such as those of *Fasciola* or infertile *Ascaris* eggs are not efficiently concentrated with this technique [20].

**Mc Master Technique:** This technique is used for the identification and quantification of the number of parasitic elements per gram of feces: eggs per gram (epg), oocysts per gram (opg), cysts per gram (cpg), larvae per gram (lpg). The specimen can be fresh or fixed stool. This test uses a special microscope slide with a grid, which makes counting easier.

**Mini-FLOTAC Technique:** This is a logical evolution of the FLOTAC conceived to perform very accurate multivalent, qualitative and quantitative diagnosis of parasitic elements (eggs, larvae, oocysts and cysts) in fecal samples, it is particularly appropriate for resource constrained settings. The Fill-FLOTAC, conversely, is a closed system designed to facilitate the performance of the first four consecutive steps of the Mini-FLOTAC techniques: fecal sample collection and weighing, homogenization, filtration, and filling of the Mini-FLOTAC chambers [21].

The Sedimentation technique, in this technique, parasites are sedimented by force (gravity and centrifuge) using formalin with some agents like ether, diethyl ether, acetone, gasoline and others for this technique, because concentrate a wide range of parasites with saving their morphology [22].

**Baermann Technique:** this technique specific to make the concentration and detection of larvae of straglyoides species and trophozoites of *Balantidium* species.

### Culture of Larval Stage

This technique directly sterno intestinal Nematodes and include; Harada-Mori filter paper strip culture; This technique used to identify infection by Hookworm, Straglyoides Stercorales and *Trichostrongylus* species.

Koga-agar plate technique; This technique especially for those patients whose suffer of immune compromise status, and considered to be the most efficient method for the detection of *S. stercorales* larvae.

### Cellophanfaecal Thick Smear

*Kato-Katz Technique* of soil-transmitted helminthes is the most important in the cellophanfaecal thick smear, and it is a diagnostic method recommended for monitoring large-scale treatment program implemented for control of soil-transmitted helminthes infections, it is a simple format and ease for use in the fields.

### Staining Procedures For protozoa in Stool

The staining smear of stool includes many types of permanent stains for stool smears with different solutions. The purpose is for teaching:

**Trichrom Stain:** It is a very good stain for fresh and unpreserved stool. SAF and formalin preserved stool sample can give good result smears. SAF and formalin preserved stool samples can give good results.

**Iron-haematoxylin Stain:** it is a very good stain for fresh and unpreserved stool

**Modified Ziehl-Neelsen Stain:** it is an acid-fast stain for detection of cryptosporidium, cyclospora and other coccidian infections [23].

### Advantages

All different intestinal parasites diagnosis techniques described with advantage according to the technique. Wet preparation (saline and iodine) described by detection of protozoan active forms and the high number of parasite elements. In concentration techniques, the advantages are detection of scanty number of intestinal parasites elements (cyst, eggs, larvae and oocyst). Culture and cellophane smear techniques, the both helpful in diagnosis of specific intestinal helminthes elements, whereas permanent stains are good for saving cyst, larvae, eggs and oocyst for long period for teaching and researches.

### Disadvantages

The most disadvantage in wet mount smear (saline and iodine) is missing of low amount of parasite elements (larvae, cyst, oocyst and eggs). All concentration techniques disadvantage is losing motile stages (trophozoites of protozoa).

### Rational

Fecal concentration has become a routine procedure as a part of complete parasite examination. It allows the detection small of organisms that may be missed by using only direct wet smear.

The formalin-ether concentration technique is commonly used in laboratories to concentrate parasite eggs, cyst, and larvae in stool specimens.

Unfortunately, diethyl ether, an essential component in the formal ether technique is extremely flammable, is highly volatile, produce anesthetic vapor and forms explosive peroxides when exposed to light.

To overcome these disadvantages, several other chemicals have been evaluated as substitutes for diethyl ether like ethyl acetate, tween, acetone, gasoline and benzene. Among these solvents, benzene has been rated as safe in comparison to the ether effect on health and has also an equivalent detection rate of parasites. This study aimed at evaluating the efficiency of formalin-benzene concentration method as newly introduced technique in comparison with the routinely used formalin-ether concentration technique for the detection of parasites [23].

### The Objectives

#### General Objective

- To made assessment and evaluation of intestinal parasites concentration (sedimentation) techniques in one of jebelaulia area countryside depending on WHO standards for intestinal parasites diagnosis.

#### Specific Objective

- To determine the best quality among the three solvents used for concentration sedimentation technique.
- To compare benzene as new solvent with ether and gasoline in detection of intestinal parasites.

### Materials and Method

#### Materials

#### Equipments and Reagents

All apparatus and laboratory equipment, chemicals, kits and consumables, and computer application programs used in this study are indicated in Tables 2.1, 2.2, and 2.3 respectively

**Table 1**

Centrifuge	
Conical tube with screw tap	USA
Cover slip	
Microscope	Olympus; Japan
Microscopic slides	
Stool container	
Votex	
Wiarseve	

**Table 2: List of Chemicals and Reagents**

Name	Suppliers
Formalin	
Diethyl ether	08181;Sertmenat SPAIN
Gasoline	Gadara oil energy
Benzene	
Logal's iodine	

**Table 3: List of Computer Application Programs and Software**

Name	Suppliers
Statistical package for the social science(SPSS),version	

## Preparation of solutions

### Formalin 10%

Formaldehyde. Formaldehyde (HCHO), also called methanal, an organic compound clear and colorless appearance, the simplest of the aldehydes, used in large amounts in a variety of chemical manufacturing processes. It is produced principally by the vapor-phase oxidation of methanol and is commonly sold as formalin, solution is 40% with widely used in biology laboratories and for storing dead biological specimens for long period (even years). It therefore, acts as a good preservative and an antiseptic. Formaldehyde is a highly toxic systemic poison if it absorbed well by inhalation. The vapor is a severe respiratory tract and skin irritant and may cause dizziness or suffocation. Contact with formaldehyde solution may cause severe burns to the eyes and skin. Formalin used as a preservative of parasitic elements (eggs, larvae, cysts and oocysts) in different concentration techniques [24].

### Diethyl Ether

Diethyl ether appears as a clear colorless liquid with an anesthetic odor. Flash point. Molecular Weight: 74.12 g/mol Molecular Formula: C<sub>4</sub>H<sub>10</sub>O or (C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>O. Ethyl ether H<sub>3</sub>CH<sub>2</sub>-O-CH<sub>2</sub>CH<sub>3</sub> is a Bromated base because can accept a proton from a strong acid HX giving the conjugate base X<sup>-</sup> and the conjugate acid H<sub>3</sub>CH<sub>2</sub>-OH<sup>+</sup>-CH<sub>2</sub>CH<sub>3</sub> Ethyl ether, also called diethyl ether, well-known anesthetic, commonly called simply ether, an organic compound belonging to a large group of compounds called ethers; its molecular structure consists of two ethyl groups linked through an oxygen atom, as in C<sub>2</sub>H<sub>5</sub>OC<sub>2</sub>H<sub>5</sub>. Diethyl ether is produced through the combination of ethanol and sulfuric acid, and has a few major uses. In parasitological laboratories, used as golden standard agent in different concentration techniques with other chemicals for detection of parasite's elements [24]. (NIOSH/OSHA - Occupational Health Guidelines for Chemical Hazards).

## Gasoline

It is a volatile flammable liquid hydrocarbon mixture used as a fuel especially for internal combustion engines and usually blended from several products of natural gas and petroleum.

The bulk of a typical gasoline consists of a homogeneous mixture of small, relatively lightweight hydrocarbons with between 4 and 12 carbon atoms per molecule (commonly referred to as C<sub>4</sub>-C<sub>12</sub>) and is mixture of many different hydrogen- and carbon- containing chemicals (hydrocarbons). A typical gasoline mixture contains about 150 different hydrocarbons, including butane, pentane, isopentane and the BTEX compounds (benzene, ethyl benzene, toluene, and xylenes). Also, it mixture of paraffin (alkanes), olefins (alkenes) and cycloalkanes (naphthenic) Gasoline, also spelled gasoline, also called gas or petrol, mixture of volatile, flammable liquid hydrocarbons derived from petroleum and used as fuel for internal-combustion engines. It is also used as a solvent for oils and fats [25].

## Benzene

Benzene is an organic chemical compound with the molecular formula C<sub>6</sub>H<sub>6</sub>. It is a colorless or light-yellow liquid at room temperature. It has a sweet odor and is highly flammable. The benzene molecule is composed of six carbon atoms joined in a ring with one hydrogen atom attached to each. As it contains only carbon and hydrogen atoms, benzene is classed as a hydrocarbon. Benzene causes harmful effects on the bone marrow and can cause a decrease in red blood cells, leading to anemia. It can also cause excessive bleeding and can affect the immune system, increasing the chance for infection [26].

Benzene has 6  $\pi$  electrons. Its first 2  $\pi$  electrons fill the lowest energy orbital, and it has 4  $\pi$  electrons remaining. These 4 fill in the orbital's of the succeeding energy level. Notice how all of its bonding orbital are filled, but none of the anti-bonding Orbital's have any electrons [27]

## Lugol's Iodine Solution

It was developed in 1829 by the French physician Jean Guillaume August Lugol, initially as a cure for tuberculosis. It is a solution of elemental iodine (5%) and potassium iodide (KI, 10%) together with distilled water. It has been used as a disinfectant, a reagent for starch detection in organic compounds, in histological preparations, in dental procedures and in diagnosis of cervical cell alterations, the Already in the 1920s LS was given as a pre-treatment to thyroid surgery. By that time iodine solution became the standard pre-operative treatment in patients with Graves' disease (GD). Iodide treatment could also be given as a saturated solution of potassium iodide (SSKI) or tablets. Radiopaque cholecystographic agents such as iopanic acid containing iodide has also been used previously, although nowadays their use is restricted. These agents are also potent inhibitors of type 1 and type 2 deiodinases, blocking the conversion of T<sub>4</sub> to T<sub>3</sub> and rT<sub>3</sub> to T<sub>2</sub>. Iodine solution used in microbiological and parasitological laboratories for giving a shin element such as bacteria, eggs, larvae, cysts and oocysts [28].

## Statistical Analysis

Statistical package for social sciences (SPSS ver. 22) was used for data entry and descriptive analysis. Mc Namur test was used to examine the association between the three diagnostic tests and the gold standard test. Test was considered significant, when P. value is less than 0.05. Sensitivity, Specificity, Positive predictive value and Negative predictive value were calculated using Medcalc online calculator [28]. The following formulas was used for analysis calculation:

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positives} + \text{False negatives}}$$

$$\text{Specificity} = \frac{\text{True negatives}}{\text{True negatives} + \text{False positives}}$$

$$\text{Positive predictive value } \frac{1}{4} = \frac{\text{True positives}}{\text{True positives} + \text{False positives}}$$

$$\text{Negative predictive value } \frac{1}{4} = \frac{\text{True negatives}}{\text{True negatives} + \text{False negatives}}$$

## Methodology

### Study Design

Descriptive cross-sectional study.

### Study Duration

The study was conducted from December 2018 to April 2019.

### Study Area

This study was administered at Jebel Aulia area Southern part of Khartoum state, the map between latitudes 15-14-28 degree North and longitudes 32-29-59 East. Bordering North White Nile state and North Al-Jazeera state.

### Sampling

#### Sampling Size

One hundred and two (102) stool samples are collected at Al-mal martial hospital in Jebel Awlia, bordering with indiscriminate abodes, were subjected in the study.

Sampling Technique

Randomize technique.

### Data Collection

No data collection.

### Data analysis

Results obtained in this study were wickerwork and transformed to electronic form using Statistical Package for Social Sciences (SPSS) software.

### Sampling Methods

The methods used for detected intestinal parasite elements (eggs, cyst, larvae and oocyst) were include four.

### Laboratory Investigations

#### Wet Mount Preparation Technique

A drop of well mixed preserved stool sample with formalin 10% was placed on microscopic slide and cover with cover slip, and pressed gently to avoid air puples. The preparation was examined under microscope using 10X objective with 10X eye piece with condenser iris closed sufficiently and the 40X objective with 10X

eye piece were used to identify the cyst, eggs, larvae and oocyst of the parasites.

#### Formal- Ether Concentration Technique

A one ml of well mixed preserved stool sample placed into conical tube with scrow tap and six ml of formalin 10% was added, then three ml of diethyl ether was added. The tube was capped and mixed well using votex machine. Immediately, the mixture was centrifuged at 2000g for one munit. Four layers were formed, a small amount of sediment (containing the parasites elements) in the bottom of the tube, a layer of formalin on top of sediment, a plug of stool debris on formalin layer and a layer of diethyl ether at the top.

The three layers were discharged and the all sediment was placed on slide and cover by cover slip, then examined under microscope using objective with the condenser iris closed sufficient to give good contrast. The 40X objective was used to identify the cyst, eggs, larvae and oocyst were present. A one small drop of iodine was added under cover slip to confirm their identification.

#### Formal- Gasoline Concentration Technique

A one ml of well mixed preserved stool sample placed into conical tube with scrow tap and six ml of formalin 10% was added, then three ml of gasoline was added. The tube was capped and mixed well using votex machine. Immediately, the mixture was centrifuged at 2000g for one munit. Four layers were formed, a small amount of sediment (containing the parasites elements) in the bottom of the tube, a layer of formalin on top of sediment, a plug of stool debris on formalin layer and a layer of gasoline at the top.

A sowab of cotton wool around wooden stick was used to remove the gasoline layer avoiding traces of gasoline oil.

The three layers were discharged and the all sediment was placed on slide and cover by cover slip, then examined under microscope using objective with the condenser iris closed sufficient to give good contrast. The 40X objective was used to identify the cyst, eggs, larvae and oocyst were present. A one small drop of iodine was added under cover slip to confirm their identification.

#### Formal- Benzene Concentration Technique

A one ml of well mixed preserved stool sample placed into conical tube with scrow tap and six ml of formalin 10% was added, then three ml of benzene was added. The tube was capped and mixed well using votex machine. Immediately, the mixture was centrifuged at 2000g for one minute. Four layers were formed, a small amount of sediment (containing the parasites elements) in the bottom of the tube, a layer of formalin on top of sediment, a plug of stool debris on formalin layer and a layer of gasoline at the top.

The three layers were discharged and the all sediment was placed on slide and cover by cover slip, then examined under microscope using objective with the condenser iris closed sufficient to give good contrast. The 40X objective was used to identify the cyst, eggs, larvae and oocyst were present. A one small drop of iodine was added under cover slip to confirm their identification.

### Results

Out of 102 stool specimens examined, 36 cases were found to be positive for different parasites. Formal-ether, formal-gasoline, formal benzene, and direct wet preparation detected 33, 36, 30 and 25 positive cases of the total specimens respectively with  $p > 0.05$ . In this study benzene, gasoline and diethyl ether were all good

in maintaining characteristic morphology however, benzene was considerably equal to gasoline and diethyl ether in they sensitivity (i.e. the mean number of detected parasites/gm of stool),Gairdia lambilia cyst was fewer seen by formal-ether.

**Conclusion**

For safety, low hazard, availability, low cost, and moderate sensitivity of benzene, this new method should be used as an alternative choice for formal-ether concentration method which is considered the gold standard.

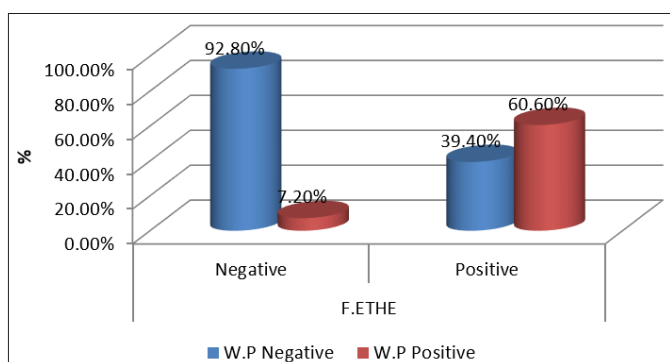
P > 0.05

**Table 4: Comparison between Formal-ether and wet preparation quality for detection of intestinal parasites**

F.ETHE	W.P		Total
	Negative	Positive	
Negative	64	5	69
	92.80%	7.20%	100.00%
Positive	13	20	33
	39.40%	60.60%	100.00%
Total	77	25	102
	75.50%	24.50%	100.00%

**Comparison parameters for Formal-ether and wet preparation quality**

Statistic	Value	95% CI
Sensitivity	60.61%	42.14% to 77.09%
Specificity	92.75%	83.89% to 97.61%
Positive Predictive Value (*)	80.00%	62.21% to 90.67%
Negative Predictive Value (*)	83.12%	76.24% to 88.31%
Accuracy (*)	82.35%	73.55% to 89.19%



**Figure 1: Detection of Intestinal Parasites by Formal-Ether and Wet Preparation**

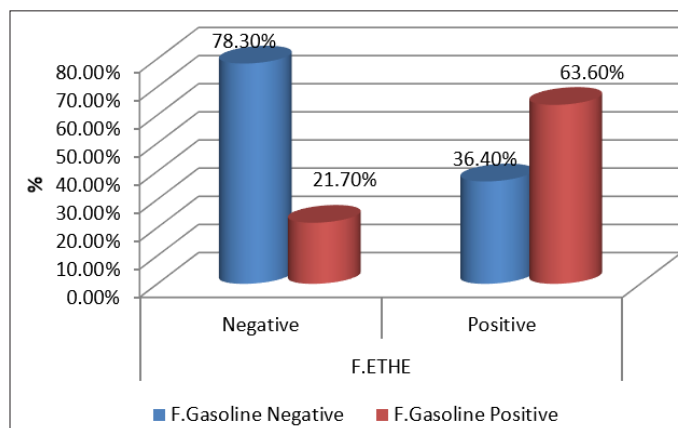
**Table 5: Comparison between Formal-ether and gasoline quality for detection of intestinal parasites**

F.ETHE	F.Gasoline		Total
	Negative	Positive	
Negative	54	15	69
	78.30%	21.70%	100.00%
Positive	12	21	33
	36.40%	63.60%	100.00%
Total	66	36	102
	64.70%	35.30%	100.00%

P > 0.05

**Comparison parameters for Formal-ether and gasoline quality**

Statistic	Value	95% CI
Sensitivity	63.64%	45.12% to 79.60%
Specificity	78.26%	66.69% to 87.29%
Positive Predictive Value (*)	58.33%	45.51% to 70.12%
Negative Predictive Value (*)	81.82%	73.81% to 87.79%
Accuracy (*)	73.53%	63.87% to 81.78%



**Figure 2: Detection of intestinal parasites by Formal-Ether and Gasoline**

**Table 6: Comparison between Formal-Ether and Benzene Quality for Detection of Intestinal Parasites**

F.ETHE	F.BENZ		Total
	Negative	Positive	
Negative	58	11	69
	84.10%	15.90%	100.00%
Positive	14	19	33
	42.40%	57.60%	100.00%
Total	72	30	102
	70.60%	29.40%	100.00%

P > 0.05

### Comparison parameters for Formal-ether and benzene quality

Statistic	Value	95% CI
Sensitivity	57.58%	39.22% to 74.52%
Specificity	84.06%	73.26% to 91.76%
Positive Predictive Value (*)	63.33%	48.27% to 76.18%
Negative Predictive Value (*)	80.56%	73.32% to 86.20%
Accuracy (*)	75.49%	65.98% to 83.47%

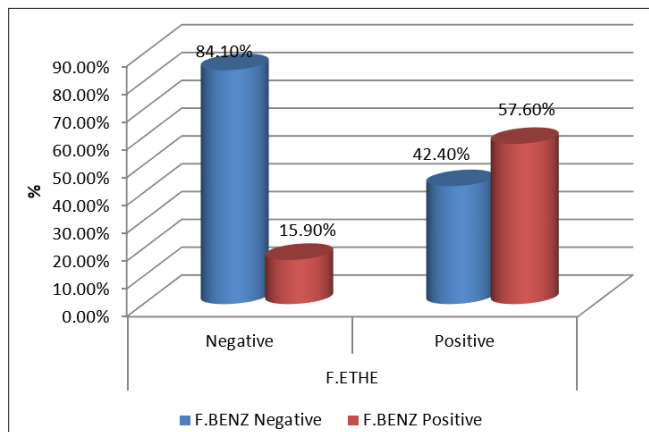


Figure 3: Detection of Intestinal Parasites by Formal-Ether and Benzene

### Definitions

- **Sensitivity:** probability that a test result will be positive when the disease is present (true positive rate).=  $a / (a+b)$
- **Specificity:** probability that a test result will be negative when the disease is not present (true negative rate).=  $d / (c+d)$
- **Positive predictive value:** probability that the disease is present when the test is positive.
- **Negative predictive value:** probability that the disease is not present when the test is negative.
- **Accuracy:** overall probability that a patient is correctly classified.= Sensitivity  $\times$  Prevalence + Specificity  $\times$  (1 - Prevalence)

### Discussion

Detection of intestinal parasites using microscopic examination is a good recommended laboratory technique that is widely used for examining stool samples by different procedures in different public and private laboratories of the world. Assessment of the competency of concentration techniques is consequential in find the accurate diagnostic techniques to provide adequate patient care, identification of the environmental agent responsible for the disease of intestinal parasites [24].

The standardized suspension specimen carefully considered free of parasites, but it contained fresh fecal material with some of which had mucus, vegetables and meat fibers, and other debris typical of stool specimens encountered in parasitological laboratories for parasite examination. We analyzed the efficiencies of wet preparation and three sedimentation techniques applied for the diagnosis of intestinal parasites in the specimens previously identified as positive or negative t for detection of intestinal parasites [24].

A concentration procedure should be performed as a routine part of a complete examination for parasites. Concentration permits the detection of organisms present in small numbers: these may be missed by using direct wet mounts. Organisms that can generally be identified using a concentration procedure include: helminthes eggs and larvae; cysts and oocyst of protozoa [3].

### Conclusion

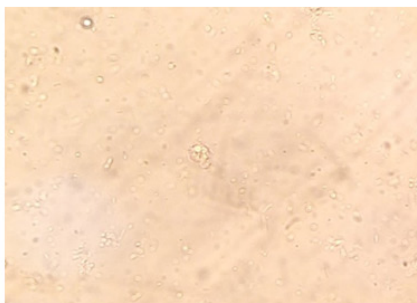
This study concluded that identification and diagnosis of intestinal parasites via high quality, must be included in concentration techniques as perpetuity to obtain and ensure best diagnosis.

### Recommendations

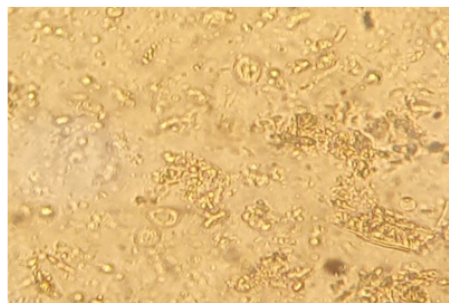
- This study recommended that
- To considering benzene as one of chemicals using for stool concentration (sedimentation) techniques to identify intestinal parasites, it needs more work.
  - Benzene very flammable and volatile, so handling should be careful.

### Appendix

#### Wet preparation



E. histolytica cyst



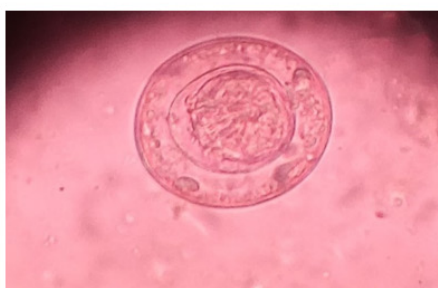
G. lamblia cyst



E. vermicular eggs

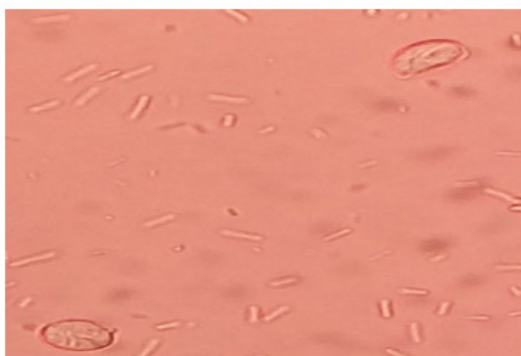


Sch. Mansoni egg

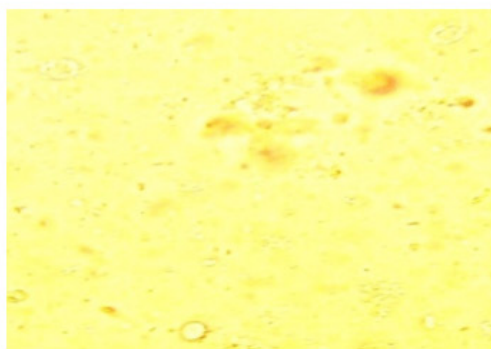


H. nana egg

### Formal Ether



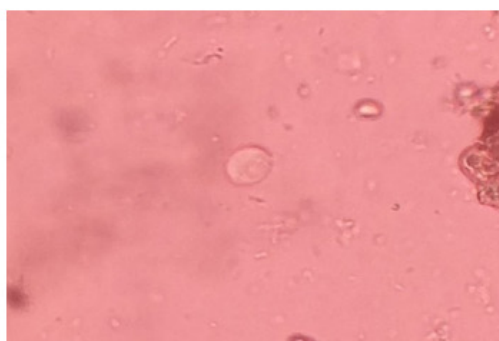
G.liamblia cyst



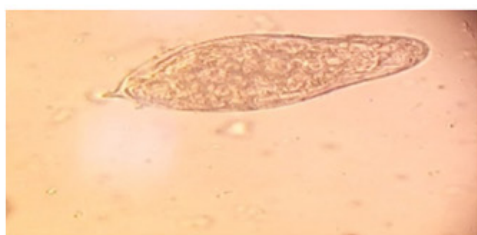
Iodine G.L cyst



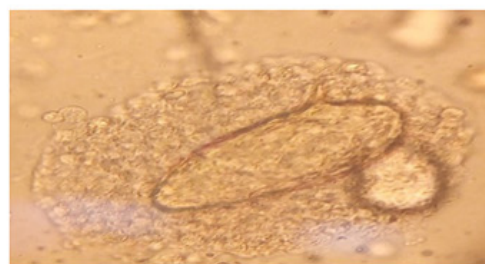
E. vermiculer eggs



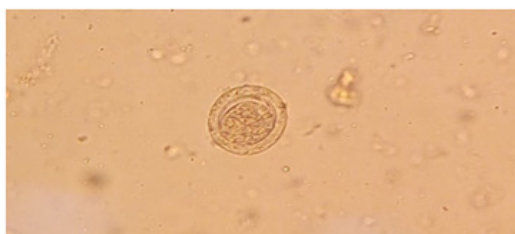
Iodine G.L cyst



Sch. Heamatobium egg

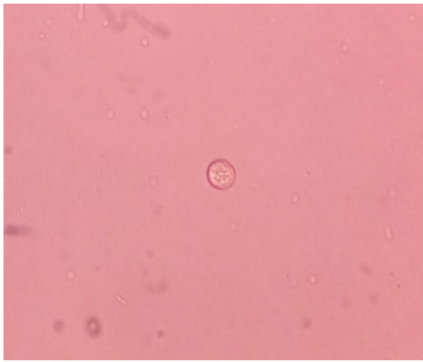


Sch. Mansoni egg

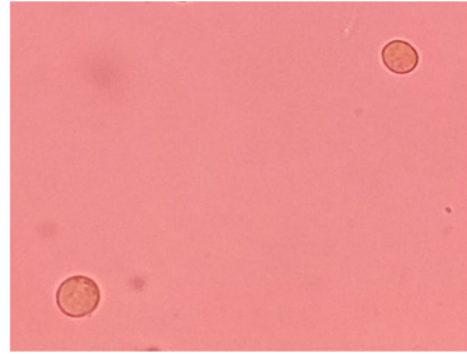


H. nana egg

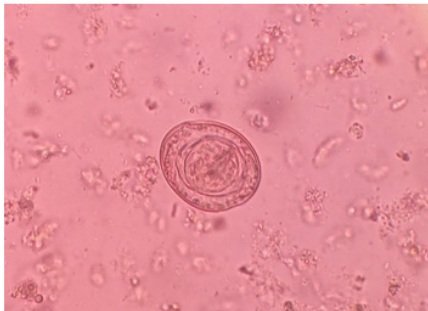
### Formal benzene



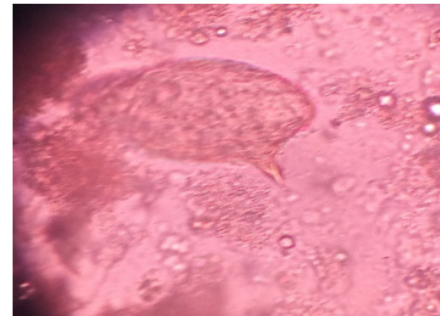
E.histolytica cyst



G.liamblia cyst



H. nana egg

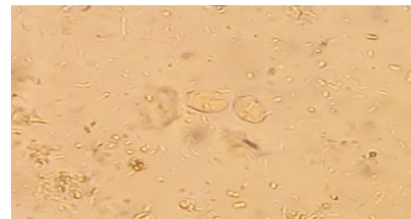


Sch. Mansoni egg

### Formal Gasoline



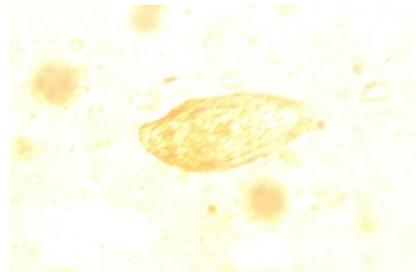
E.histolytica cyst



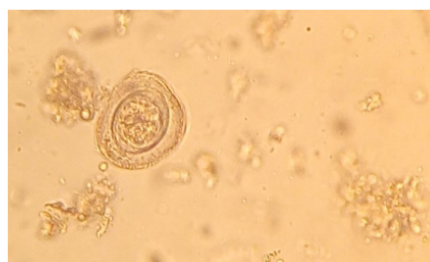
G.liamblia cyst



Sch. mansoni egg



Idoine Sch. mansoni egg



H. nana egg

### Equipment:



Conical tubes



centrifuge + vortex machine



Concentration chemical



Stool samples



Microscope

### References

1. Bineshlal Y, Thiyagarajan A, Jayakumar S (2015) Comparison of different concentration techniques of the stool examination for detecting intestinal parasites. *Research Journal of Pharmaceutical, Biological Chemical Sciences* 6: 873-878.
2. WHO (2012).
3. Kalaivani Ramakrishnan, Arunava Kali, Pravin Charles MV, Seetha Kunigal S (2016) Comparative Evaluation of Three Stool Concentration Techniques in the Diagnosis of Intestinal Parasitic Infections. *Int J Curr Microbiol App Sci* 5: 299-304.
4. Feleke Moges, Yeshambel Belyhun, Moges Tiruneh, Yenew Kebede, Andargachew Mulu1, et al. (2010) Comparison of formal-acetone concentration method with that of the direct iodine preparation and formal-ether concentration methods for examination of stool parasites. *Ethiop J Health Dev* 24: 148-153.
5. Elamin Abdelkarim Elamin (2015) A comparison between the efficiency of formal gasoline concentration technique and other techniques used for the detection of intestinal parasites. *International Journal of Preclinical & Pharmaceutical Research* 6: 91-94.
6. Pakdad K, Nasab SDM, Damraj FA, Ahmadi NA (2018) Comparing the efficiency of four diagnostic concentration techniques performed on the same group of intestinal parasites. *Alexandria Journal of Medicine* 54: 495-501.
7. World Health Organization (2019) Bench aids for the diagnosis of intestinal parasites. <https://www.who.int/publications/i/item/9789241515344>.
8. Ahmadi NA, Damraj FA (2009) A field evaluation of formalin-gasoline technique in the concentration of stool for detection of intestinal parasites. *Parasitol Res* 104: 553-557.
9. WHO (2004).
10. WHO (2014).
11. Feleke Moges, Yeshambel Belyhun, Moges Tiruneh, Yenew Kebede, Andargachew Mulu1, et al. (2010) Comparison of formal-acetone concentration method with that of the direct iodine preparation and formal-ether concentration methods

- for examination of stool parasites. *Ethiop J Health Dev* 24: 148-153.
12. World Health Organization (2004) Training Manual on Diagnosis of Intestinal Parasites based on the WHO Bench Aids for the diagnosis of intestinal parasites Schistosomiasis and Intestinal Parasites Unit Division of Control of Tropical Diseases. [https://iris.who.int/bitstream/handle/10665/63790/WHO\\_CTD\\_SIP\\_98.2.pdf?sequence=1](https://iris.who.int/bitstream/handle/10665/63790/WHO_CTD_SIP_98.2.pdf?sequence=1).
  13. (2012) An evaluative study of the use of gasoline as an alternative to ether in the concentration technique for detecting intestinal parasites. *Basra Research Journal (Scientific) Issue*. <http://www.basra-sciencejournal.org>.
  14. Jamsai Suwansaksri, Suwannee Nithiuthai, Viroj Wiwanitkit, Suphan Soogarun, Pennapa Palatho (2002) The Formol-Ether Concentration Technique for Intestinal Parasites: Comparing 0.1 N Sodium Hydroxide with Normal Saline Preparations. *Southeast Asian J Trop Med Public Health* 33: 97-98.
  15. V Wirkom, R Tata, M Agba, G Nwobu, R Ndze, et al. (2007) Formal-petrol stool concentration method (Wirkom-Tata's stool concentration method): A Cheap Novel Technique for Detecting Intestinal Parasites in Resource-Limited Countries. *The Internet Journal of Tropical Medicine* 5.
  16. Alfa Omar Diallo (2004) *Microbiology Recall*. Goodreads [https://www.goodreads.com/book/show/1678088.Microbiology\\_Recall](https://www.goodreads.com/book/show/1678088.Microbiology_Recall).
  17. David Greenwood, Richard Slack Will Lrving (2000) *Medical microbiology Guide to microbial infections Pathogenesis, immunity, laboratory diagnosis and control*. Eighteenth Edition <https://books.google.co.in/books?id=eeuDjsA66CAC&printsec=frontcover#v=onepage&q&f=false>.
  18. Arora DR, Brij Bala Arora (2010) *Medical Parasitology*. Central Library Vidyasagar University <https://libnet.vidyasagar.ac.in/cgi-bin/koha/opac-detail.pl?biblionumber=25878>.
  19. Gunner Damgard Nielsen, Uffe Kristiansen, Lea Hansen, Yves Alarie (1982) Irritation of the upper airways from mixtures of cumene and n-propanol. Springer Nature Link <https://link.springer.com/article/10.1007/BF00570142>.
  20. Monica Cheesbrough (1998) District Laboratory practice in tropical counties. <https://www.medbox.org/preview/5255d6e1-05d4-41a9-beb2-02b60e695ecc/doc.pdf>.
  21. Robert AA, Adrea KR (1987) Standard chemical thermodynamic properties of polycyclic aromatic hydrocarbon and their isomer groups I. Benzene series. Department of chemistry Massachusetts institute of technology, Cambridge Massachusetts 02139 <https://www.nist.gov/system/files/documents/srd/jpcrd341.pdf>
  22. Free statistical calculators Diagnostic test evaluation calculator. [https://www.medcalc.org/en/calc/diagnostic\\_test.php](https://www.medcalc.org/en/calc/diagnostic_test.php).
  23. Bench aids for diagnosis of intestinal parasites /second edition/2019.
  24. (NIOSH/OSHA - Occupational Health Guidelines for Chemical Hazards) <https://www.cdc.gov/niosh/docs/81-123/default.html>.
  25. Benzene – Wikipedia. <https://en.wikipedia.org/wiki/Benzene>.
  26. (17.7: The Criteria for Aromaticity - Hückel's Rule). [https://chem.libretexts.org/Bookshelves/Organic\\_Chemistry/Map%3A\\_Organic\\_Chemistry\\_\(Smith\)/15%3A\\_Benzene\\_and\\_Aromatic\\_Compounds/15.07%3A\\_The\\_Criteria\\_for\\_Aromaticity\\_-\\_Huckels\\_Rule](https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Map%3A_Organic_Chemistry_(Smith)/15%3A_Benzene_and_Aromatic_Compounds/15.07%3A_The_Criteria_for_Aromaticity_-_Huckels_Rule).
  27. Calissendorff J, Falhammar H (2017) Lugol's solution and other iodide preparations: perspectives and research directions in Graves' disease. *Endocrine* 58: 467-473
  28. [https://www.medcalc.org/calc/diagnostic\\_test.php](https://www.medcalc.org/calc/diagnostic_test.php).