

Experimental Studies on Stool Microscopic Analysis by YBIO TeStool Routine and Concentration Test

Dhinesh Kumar.T and Elango M.M

Head of the R and D Department, Yuvraj Biobiz Incubator India PVT LTD, Chennai, India.

Chairman (CMD), Yuvraj Biobiz Incubator India PVT LTD, Chennai, India.

dr.dhinesh@ybio.in

ABSTRACT

Around 3.5 billion people were affected and 450 million people developed illness because of gastrointestinal parasitic infection. Due to this parasitic infection, the nutritional status, physical development, mental function, cognitive behaviour and verbal ability gets affected in children. Overall, stool samples of 100 numbers were purchased from different Hospitals (Christian Medical College, Vellore, MIOT Hospital, Chennai and JIPMER, Pondicherry) and few Clinical Labs (Winpath Labs, Chennai). Among the 100 samples, 10 numbers were positive which contained *Giardia spp*, *Ascaris spp* and Hookworms were also present in it, 20 samples were occult blood positive and 70 samples were of Health check-up samples (Unknown). All the suspected and positive samples were subjected to both procedures such as Stool Routine Test (Wet Mount Method, YBIO TeStool – Stool Routine Test kit) and Stool Concentration Test (Concentration - Sedimentation Method, YBIO TeStool – Stool Concentration Test Kit and Mini-Parasep SF). All the stool tests like RBC, Pus Cells, Cyst, Ova, Helminths and Parasites etc., and culture test (isolation of bacteria by spread plate method) recommended to use YBIO TeStool-Stool routine test kit and observation of debris were less than direct wet mount method. Finding of Cyst, Ova, Helminths and Parasites detection test were recommended to be done by both Mini-Parasep SF and YBIO TeStool-Stool concentration test kit but this test not applicable to find RBC, Pus Cells, Fibres and Culture test. These commercial kits showed more effective results in both stool routine and stool concentration test than traditional method.

Keywords: Parasites, Stool Analysis, Stool Concentration Test, TeStool Kit

INTRODUCTION

In the developing countries, the major proportions of people are lack of water, with poor sanitation and hygiene conditions majorly presents them for diseases and also leads to mortality. Due to the above issues, they are deprived of their rights to healthy and dignified lives. In the world wide, the infection caused by intestinal parasites exhibited high impact on the human health. The nutritional status, physical development, mental function, verbal ability and inhibition control aspects of cognitive behaviour in children is affected largely by parasitic infections. (Okay et. al., 2004 and Tripathy et. al., 1973) In the tropical countries, the infection of intestinal parasites is an overwhelming problem. Around 3.5 billion people were affected and 450 million people have become ill (WHO 1998). The gastrointestinal parasite infection mostly occurs in children and immune deficit patients. In the diagnosis of parasite infection, stool sample are used to find microscopic organisms such as trophozoites and cysts, helminth eggs, and larvae. (Vinay Khanna et. al., 2018)

In the diagnosis, mostly preferred method is formo-ether method (Paugam et. al., 2016) which derived from riddly-allen method (Manser et. al., 2016). It is known as sedimentation method. Usually, they are using direct wet method, sedimentation method and floatation method. When compared to all the results, sedimentation technique showed goods results for finding parasites but the RBC, WBC and Culture test cannot be done. The direct wet mount test is used to detect the white blood cells which help to fight against the foreign particles. The content of leukocytes when present in high numbers in the suspected stool indicates Inflammatory bowel disease or bacterial infection.

Mostly, the stool testing was done by physical factors (Kasirga E, 2019) such as color (Tawny), Mucus (High level present is abnormal), Consistency, and Quantity and Microscopic analysis such as RBC, pus cells, helminths, cyst, ova and Culture Test (Humphries RM, and Linscott AJ. 2015). If the stool sample looks watery stage (dysentery) then it is caused by *Shigella* spp (*S.dysenteriae*, *S.flexneri*, *S.boydii* and *S.sonnei*), *Escherichia coli* (EIEC and EHEC), *V.parahemolyticus*, *Campylobacter jejuni* and *Salmonella* spp and the stool sample of diarrhoea which is caused by Gram positive bacteria such as *Staphylococcus aureus*, *Clostridium perfringens*, *Clostridium difficle*, *Bacillus cereus* and Gram negative bacteria such as *Vibrio cholerae*, *Vibrio parahemolyticus*, other halophlic vibrios, *Escherichia coli*(*ETEC*, *EPEC*) *S.enteritidis*, *S.typhimurium*, *Shigella* spp, *Campylobacter jejuni* and *Yersinia enterocolitica*. Some of Protozoa like *Entamoeba histolytica*, *Giardia lamblia*, Intestinal Coccidian Parasites such as *Cryptosporidium parvum*, *Cyclospora*, *Isospora*, *Balantidium Coli*, Helminthes (Nematodes: *Ascaris lumbricoides* and *Trichuris trichuria*) and Hookworms (*Ancylostoma duodenale*, *Nectar americans*, *Strongyloides stercoralis* *Taenia* spp *T. saginata* *T.solium* *Hymenolepsis nana* and *Enterobius vermicularis*) also causes diarrhoea (Santosh Kotgire, 2012).

METHODOLOGY

Stool Samples

Totally, stool samples in 100 numbers were purchased from different Hospitals (Christian Medical College, Vellore, MIOT Hospital, Chennai and JIPMER, Pondicherry) and Clinical Labs (Winpath Labs, Chennai). Among the 100 samples, 10 numbers were positive which contained *Giardia* spp, *Ascaris* spp and Hookworms were also present, 20 samples were occult blood positive and 70 samples were of Health check-up (unknown) samples.

The license details are mentioned below obtained to manufacture the YBIO TeStool – Stool Routine Test and YBIO TeStool – Stool Concentration Test. Licence No. - MFG/TL/IVD/2019/000113, received from Central Drugs Standard Control Organisation (CDSCO), Directorate General of Health Services, Ministry of Health & Family Welfare, (Medical Device & Diagnostic Division), FDA Bhawan, Kotla Road, New Delhi-110002.

Stool Routine Test

In this test, Cyst, Ova, Red blood cells, White blood cells and Culture tests were performed. We followed two different procedure i.e. Traditional Method i.e., wet mount method and YBIO TeStool- Stool Routine Test for comparative studies.

Wet Mount Method

As per procedure, 0.5g - 1g of stool sample was put on the glass slide by directly, added to 0.89% or 0.9% of Sodium chloride solution (Leveck et. al., 2009). The prepared glass slide was subjected for microscopic examination for the test analysis.

YBIO TeStool – Stool Routine Test

1g of stool sample was added into the 3ml buffer container which contains 3 ml of 0.89% of sodium chloride solution continuously use nozzle with filter was put on the bottle and mixed thoroughly. The sample prepared solution was added into the centrifuge tube/serum tube at 5000RPM for 5minutes. The obtained pellet was used for microscopic examination.

Stool Concentration Test

In this test, Cyst, Ova, and Parasite were tested. We followed three different procedure.

Sedimentation Technique

Organic solvents such as Diethyl Ether and Formalin etc., and gauze filters were used in this method (Ritchie, 1948, Ridley DS and Hawgood BC, 1956, Allen AVH and Ridley DS, 1970). The commercial kits (Picture.1) such as YBIO TeStool Stool Concentration Test and Mini-Parasep SF were used.

YBIO TeStool – Stool Concentration Test

1g of stool sample was added into the 3ml buffer bottle which contained 1ml of 10% Formalin, 1ml of 1% Diethyl ether and 1ml of 0.89% of Sodium chloride solution continuously use the nozzle filter was put on the bottle and mixed thoroughly. The sample prepared solution was added into the centrifuge tube/Serum Tube at 5000RPM for 5minutes. The pellet was used for microscopic examination.

Mini-Parasep SF

The spoon full of stool sample was added in to the buffer which contained mixture of Menthol, Acetic Acid and Thymol. The tube was centrifuged at 5000RPM for 3minutes. In this method, we followed 4 major methods (Manser et. al., 2016) for this procedure i.e. Sample preparation, Emulsification, Centrifugation and Examination. The pellet was used for microscopic analysis.

RESULTS AND DISCUSSIONS:

All the samples were subjected to both procedures such as Stool Routine Test (Wet Mount Method, YBIO TeStool – Stool Routine Test kit) and Stool Concentration Test (Sedimentation Method, YBIO TeStool – Stool Concentration Test Kit and Mini-Parasep SF).

For all the two tests (Wet mount method and YBIO TeStool-Stool Routine test), Red blood cells, Pus cells, Fibres, *Ascaris spp*, (Table.1) Hookworms and *Giardia spp* were visualized under the microscope. In the direct wet mount method, all the organisms were observed but more debris were present so it affected the accuracy of the results. Duration to find out the microscopic organisms, utilized in around 25 to 45 minutes but the procedure time is very

less. The RBC and Pus cells were easily detected from the all remaining samples. In the YBIO TeStool – Stool Routine Test, we observed the presence of Red blood cells, Pus cells, Fibres, *Ascaris spp*, Hookworms and *Giardia spp* (Picture.2) easily under the microscope.

Stool routine test, when compared to traditional wet mount method, YBIO TeStool Stool routine test showed high accuracy of result and less time to find the samples with contaminants.

All the three tests (Sedimentation method, YBIO TeStool-Stool Concentration test and Mini-Parasep SF), Red blood cells and Pus cells, and Fibres were not found and *Ascaris spp*, Hookworms and *Giardia spp* were visualized under the microscope. In the sedimentation method, all the organisms were observed but more debris were present so it affected the accuracy of the results. Duration to find the samples with contaminants was around 15 to 35 minutes and the procedure time required is also high. The RBC and Pus cells were less detected in (Table.2) YBIO TeStool-Stool Concentration test.

In the YBIO TeStool – Stool Concentration Test kit, we observed all the contaminants such as *Ascaris spp*, Hookworms and *Giardia spp* (Picture.3) and were easily detected under the microscope. When compared to Sedimentation test, YBIO – TeStool – Stool Concentration Test kit showed results with high accuracy and the procedure time required is also very less. Also when compared to Mini-Parasep pellet of stool (Table.3), debris is present in fewer amounts when using YBIO – TeStool – Stool Concentration Test kit and more debris is present in sedimentation test.

CONCLUSION

We recommend to use YBIO TeStool-Stool routine test kit for Stool routine test because it shows effective results which helps to identify the Red blood cells, Pus cells, Fibres, *Ascaris spp*, Hookworms and *Giardia spp* easily than direct wet mount method. The accuracy of result obtained is also higher and procedure of this test is easily understandable. Even, the pellet of the suspected stool sample can be used for culture test without adding any reagents/solvents. Stool concentration test for parasite detection (Cyst, Ova, Helminths) is recommending to be performed by both YBIO TeStool-Stool concentration test kit and Mini-Parasep SF. Mini-Parasep SF test results showed less debris than YBIO TeStool-Stool concentration test kit under the microscope and TeStool kit showed less debris than Sedimentation method. All the stool tests (stool routine test) like RBC, Pus cells, Cyst, Ova, Parasites and culture test can be done by YBIO TeStool-Stool routine test kit and for detecting only Cyst, Ova, and Parasites tests can be performed with both Mini-Parasep SF and YBIO TeStool-Stool concentration test kit. All these methods showed effective results than traditional method.

REFERENCES

1. P. Okyay, S. Ertug, B. Gultekin, O. Owen, and E. Beser, “Intestinal parasite prevalence and related factors in school children, a Western city sample – Turkey”, *BMC Public Health*, vol. 22, Dec. 2004.
2. K. Tripathy, F. Gonzalez, and H. Lotero, “Effects of *Ascaris* infection on human nutrition”, *Am J Trop Med Hyg*, vol. 20, pp.212 – 218, 1971.
3. “World Health Organization. Control of Tropical Diseases. Geneva”, *WHO*, 1998.

4. V. Khanna, S. Sagar, R. Khanna, and K. Chawla, “A comparative study of formalin-ethyl acetate sedimentation technique and Mini Parasep solvent-free method in the rapid diagnosis of intestinal parasites”, *Trop Parasitol*, vol. 8(1), pp. 29–32.
5. A. Pauga, F. Ngamada, ED. Pécoulas, and H. Yéra Diagnosis of Intestinal Parasitoses: Comparison of Two Commercial Methods for Faecal Concentration Using a Polyparasitized Artificial Liquid Stool, *Appli Micro Open Access*, vol. 2, 2016.
6. MM. Manser, ACS. Saez, and PL. Chiodini, *Faecal Parasitology: Concentration Methodology Needs to be Better Standardised*, “PLoS Negl Trop Dis”, vol.10(4), 2016.
7. E. Kasirga, “The importance of stool tests in diagnosis and follow-up of gastrointestinal disorders in children”, *Turk Pediatri Ars*; vol. 54(3), pp. 141–148. 2019.
8. RM. Humphries, and AJ. Linscott, “Laboratory diagnosis of bacterial gastroenteritis”, *Clin Microbiol Rev*, vol. 28, 2015.
9. S. Kotgire, “Microbiological Stool Examination: Overview”. *Journal of Clinical and Diagnostic Research*. vol-6(3), pp. 503-509, 2012
10. B. Levecke, N. De Wilde, E. Vandenhoute, and J. Vercruysse, “Field validity and feasibility of four techniques for the detection of *Trichuris* in Simians: a model for monitoring drug efficacy in public health?” *PLoS Negl Trop Dis*. Vol. 3, pp. 1–5. 2009.
11. LS. Ritchie, “An ether sedimentation technique for routine stool examination”, *Bull. U.S. Army Med*, vol. 8, 1948.
12. DS. Ridley, and BC. Hawgood, “The Value of Formol-Ether Concentration of Faecal Cysts and Ova”, *J. Clin. Pathol.*; vol. 9, pp. 74–76. 1956
13. AVH. Allen and DS. Ridley. “Further observations on the formol-ether concentration technique for faecal parasites”. *J. Clin. Pathol*, vol. 23: pp. 545–546, 1970.

Tables 1: Results observation under Microscope with direct wet mount method and YBIO-TeStool Routine Test

S.No	Tests	Wet Mount Method	YBIO-TeStool Routine Test
1	<i>Ascaris spp</i>	+	+++
2	Hookworms	+	+++
3	<i>Giardia spp</i>	++	+++
4	Red Blood Cells	++	+++
5	Pus Cells	++	+++
6	Culture Test	+	++

+ - Low accuracy; ++ - Medium Accuracy and +++ - High Accuracy

Tables 2: Results observation under Microscope with Sedimentation method, Mini Parasep SF and YBIO-TeStool Concentration Test

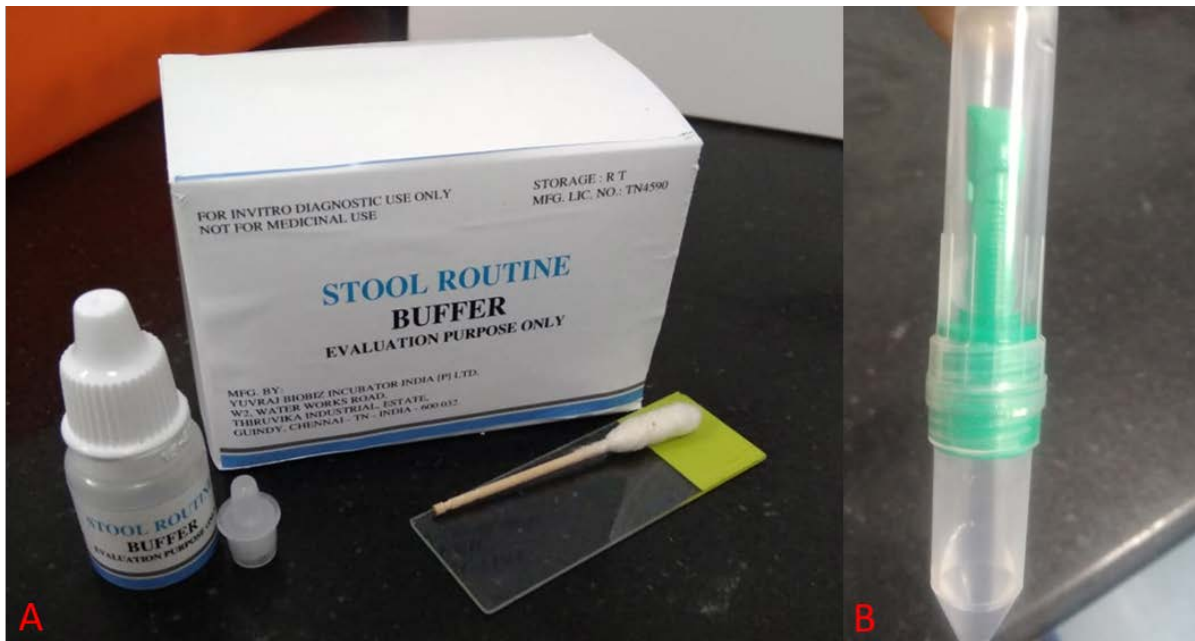
S.No	Tests	Sedimentation method	Mini Parasep SF	YBIO-TeStool Concentration Test
1	<i>Ascaris spp</i>	+	+++	+++
2	Hookworms	++	+++	+++
3	<i>Giardia spp</i>	+	+++	+++
4	Red Blood Cells	+	+++	++
5	Pus Cells	+	+++	++

+ - Low accuracy; ++ - Medium Accuracy and +++ - High Accuracy

Tables 3: Comparative Results observation under Microscope with Sedimentation method, Mini Parasep SF and YBIO-TeStool Concentration Test, YBIO-TeStool Routine Test, and Wet Mount Method

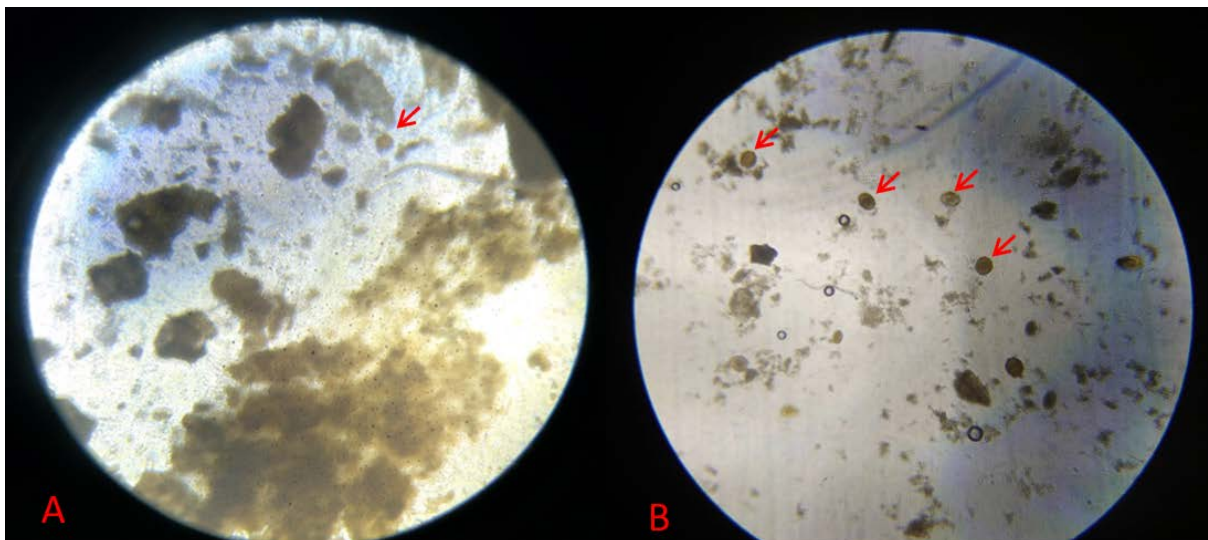
S.No	Tests	Sedimentation method	Wet Mount Method	YBIO-TeStool Concentration Test	YBIO-TeStool Routine Test	Mini Parasep SF
1	<i>Ascaris spp</i>	+	+	+++	+++	+++
2	Hookworms	++	+	+++	+++	+++

+ - Low accuracy; ++ - Medium Accuracy and +++ - High Accuracy



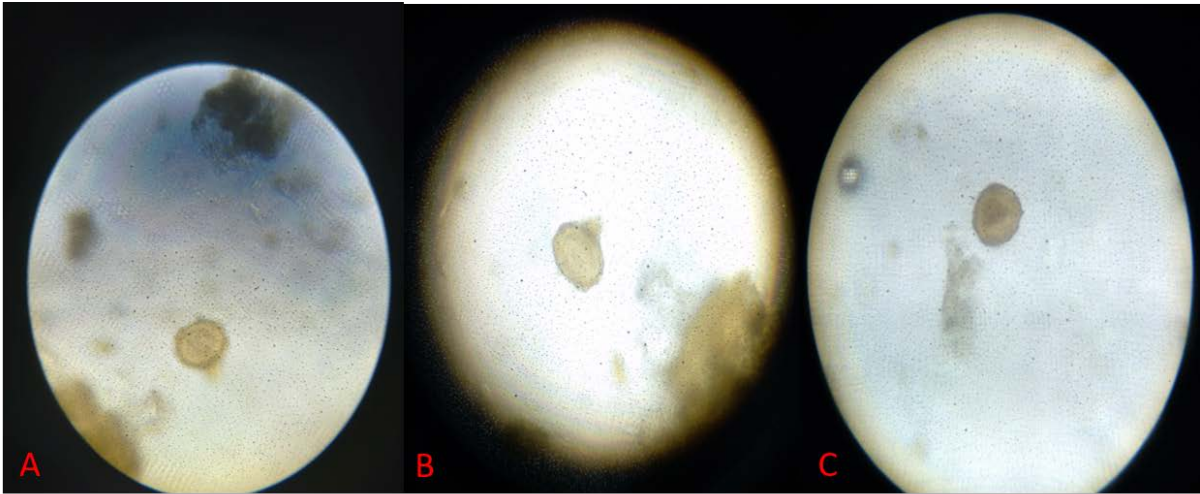
Picture-1: Commercial kits for stool testing

A-YBIO TeStool – Stool testing kit; B-Mini Parasep SF



Picture-2: Comparison results of *Ascaris* spp sample with Wet mount method and YBIO TeStool-Stool routine test

A-Direct wet mount method; B- YBIO TeStool-Stool routine test



Picture-3: Comparison results of *Ascaris spp* sample with Sedimentation method, YBIO TeStool-Stool concentration test and Mini-Parasep SF

A- Sedimentation method; B- YBIO TeStool-Stool concentration test; C- Mini-Parasep SF