Stool concentration methods:

Concentration method may be used to seen whether treatment of the parasites has been successful . To examine stool specimens from patients who do not come from an area where a particular parasite is found. Examination of direct wet mount may not detect parasite, if the number of parasite in stool is low , thus the stool should be concentrated . The concentration procedure is used when the initial wet mount examination give negative result despite the clinical symptoms of patient indicate parasitic infection because the concentration procedures separate parasites from fecal debris and increase the chances of detecting parasites when they are in small number.

Worm eggs, larvae and protozoan cysts may be recovered but protozoan trophozoites will not be seen as they are usually destroyed during the concentration procedure. Many concentration methods have been employed , all of which attempt to separate protozoan cyst and helminth eggs from the bulk of fecal matter through differences in specific gravity.

The described methods fall into two general classes:

1-Flotation techniques: involves the use of a heavy liquid that make the lighter parasites rise to the surface .

2-Sedimentation: with the various sedimentation methods , eggs and cysts , which are heavier than the suspending liquid, become concentrated in the bottom of a tube.

Fecal flotation methods

Fecal flotation methods levitate the diagnostic product of endoparasitic organisms (eggs, larvae, oocysts and cyst) in the feces by use of suspension medium with a higher specific gravity than the parasite products. Parasite eggs, cysts and oocysts are concentrated on the surface of the medium because of their lighter density.

The result is a clean preparation for microscopic examination with a minimal amount of distracting fecal debris. This method can be used to concentrate cysts, larvae and most helminthes eggs except those of *Paragonimus westermani* , *Fasciolopsis buski*, *Clonorchis sinensis* and *Diphyllobothrium latum* and other operculated eggs and also Schistosomes eggs which eggs do not float.

Saline smear showing the characteristic flask shape of the *Clonorchis sinensis* ova

Three different flotation media widely used in medical practice,

1-Saturated salt solution 2- Saturated sheather´s sucrose

3-Zinc sulfate solution.

Formal Ether Sedimentation techniques

The principle is that ether or ethyl acetate as a liquid (fatty plug) removing agent from stool and formalin as a fixative (fixation of parasites)

Advantages

1-Recover all types of worm eggs, larvae and protozoan cyst and retain their morphology (thus facilitating identification of parasite)

2-Recommended for general diagnostic laboratories because they are easier to perform and less prone to technical errors.

3-Can be used with specimens preserved in formalin, MIF, SAF

Parasep method: is a commercial kit for fecal parasite concentration , it is a rapid single use , disposable module for the clean and efficient concentration of helminth ova , protozoal cysts and oocysts. The methodology is a modification of the Ridley-Allen method.

It consists of a mixing chamber in which the feces is mixed with the 10% formalin. The ether or ethyl acetate is added (1 drop of triton-X is added to the mixture when ethyl acetate is used as it helps to break up the fecal matter) and parasep is immediately sealed by screwing the filter/ thimble sedimentation cone onto the mixing chamber. The seal is an air/liquid seal which prevents the release of biohazardous material. There is also a safety lock to ensure that the mixing chamber and filter thimble are removed together for safe disposal.

The mixture is vortexed and parasep is then inverted to allow the mixture to be filtered through the filter thimble.

Parasep is then centrifuged at 3000 rpm for 1 minute. The mixing chamber and filter thimble are unscrewed and discard .

Like the conventional Ridley-Allen sedimentation method there is a ether/ethyl acetate layer , fatty plug, formalin and sediment, the fatty plug is loosened and the supernatant discarded. The deposit is examined for ova, cysts and larvae.

Technique	Advantages	Disadvantages
Direct smear	1-Quick to prepare 2-No distortion of parasites if isotonic saline is used as diluents. 3-Only way to see live trophozoites(isotonic saline must be used as the diluents. 4-Useful for examination feces of small birds and reptiles (where trematode eggs are common)	1-Can miss parasite if concentration is too low or if too much debris or fat is present. 2-Sand, seeds or other fecal debris can make apposition of coverslip onto slide difficult 3-May take a long time to examine
Zinc Sulfate Flotation	1-Recommended procedure for most fecal exams 2-Procedure floats most helminth eggs. 3-Best method for protozoan cysts especially *Giardia*. 4-There is little debris to obscure view of parasites.	1-Procedure will not float some trematode ova and some tapeworm (Pseudophyllidian) ova 2-Unsuitable for fatty stool samples. 3-$ZnSO_4$ is expensive and a hydrometer should be used to make up the solution.
Ethyl acetate sedimentation	1-Procedure recovers all types of helminth ova, larvae and most protozoan cysts.	1-It is more difficult to perform than other techniques.
	2-It is the best technique for formalin-fixed samples and for stools with high fat content.	2-Ethyl acetate is flammable and expensive 3-There is more debris in preparation preps than in flotation preps therefore it will take longer to read.