Summary

Formalin and polyvinyl alcohol (PVA) which contains mercuric chloride have been trusted preservatives in the past. However, formalin is carcinogens and mercuric chloride is potentially hazardous to laboratory personnel and presents disposal problems.

The aim of the present study was to evaluate different techniques for the preservation and staining of intestinal protozoa in faecal specimens.

A total of 60 fresh stool samples were collected from patients; of different ages and of both sexes, attending the out-patient clinics of (Beni Suief, Abu El-Reesh and Kasr El-Aini Hospitals). These patients were complaining of various intestinal manifestations, such as diarrhoea, colic, flatulence, constipation, and /or weight loss.

Each stool sample was preserved using three different preservatives; SAF which contains formalin; PVA which contains mercuric chloride and EcoFix which is formalin- and mercuric chloride- free. Each preserved stool sample was subjected to three techniques: direct wet mount preparation, concentration using simple sedimentation and permanent staining. In permanent staining, each fixative was matched with the appropriate stain; iron-haematoxylin stain for SAF-preserved specimens, Wheatley's trichrome stain for PVA-preserved specimens and EcoStain for EcoFix-preserved specimens. Assessment of the different preservatives and the permanent stained slides was done.

The first matched set was **SAF preservative/iron-haematoxylin stain**. SAF was easy to prepare in the laboratory within a short period of time. All the reagents were available and were of low cost. SAF revealed excellent preservation of key morphologic features of the examined protozoan trophozoites and cysts even for 10 months preservation time. Moreover, it showed clear background during wet mount examination in about 93.3% that

facilitated identification of intestinal protozoa. On the other hand, SAF has poor adhesive properties, contains formalin and the pH has to be adjusted.

Concerning its matched iron haematoxylin stain (Fe-Hx), all the reagents were of low cost and the smear needs only 30 min for drying before staining. Fe-Hx stain gave good results especially for *Entamoeba spp.* trophozoites and cysts, *Blastocystis hominis* and human cells that were easily differentiated from *E. histolytica* cysts. On the other hand, Fe-Hx stain was difficult to prepare and the working solution has to be made fresh every week. The staining procedure was processed in too long time (about 1 hour). Also, it gave less adequate staining quality with flagellate cysts and trophozoites. The background contrast was too minimal to help rapid identification of intestinal protozoa

The second matched set was **PVA preservative/Wheatley trichrome stain (WT)**. PVA revealed excellent preservation of key morphologic features of the examined protozoan trophozoites and cysts even for 10 months after preservation. On the other hand, PVA was the most difficult in preparation and the most expensive. PVA showed the dirtiest background that obscured the identification of protozoan parasites in about 25% of specimens.

Wheatley's trichrome, the matched stain with PVA, was easy to prepare and needed only 30 min for preparation and have a long shelf life. WT showed good staining quality for amoeba trophozoites, flagellate cysts, *Blastocystis hominis* and human cells that were easily differentiated from *E. histolytica* cysts. The background showed an adequate contrast with the protozoan parasites that was more distinct than that obtained with iron haematoxylin stain.

Limitations of WT included that the smear had to be dried overnight. The staining procedure was processed in about 11 steps that took approximately 45min. It was expensive due to its content of chromotrope 2R.WT showed poor staining quality for flagellate trophozoites and for amoebae cysts especially *E. coli* cysts in which identification of nuclei was difficult.

The third matched set was **EcoFix/ EcoStain**. EcoFix was easy to prepare in the laboratory within a short period of time. All the reagents were available and it has the advantage of not containing mercuric chloride. EcoFix was a suitable preservative for maintaining the morphology of intestinal protozoa and for long term storage even for 10 months of preservation. The background was clear in about 86.7% that facilitated identification of the organisms. The only disadvantage of EcoFix is its content of PVA powder which made it to some extent expensive.

Concerning its matched stain EcoStain method, the staining procedure was processed in the least number of steps (8 steps) that took approximately 25 min. EcoStain demonstrated the best staining quality for flagellate cysts and trophozoites. Also, it showed good staining for amoeba trophozoites, *B. hominis* and human cells. The background provided a good contrast with protozoan parasites. On the other hand, EcoStain was difficult to prepare, expensive and the smears have to be dried overnight. The stain gave poor results with *Entamoeba* cysts especially *E. coli* cysts which showed hardly seen nuclei.

From the above mentioned data, it was concluded that EcoFix can be used as an environmentally safe alternative to the traditional formalin and PVA both in the examination of wet mount and for the preparation of permanent stained smears especially when used with EcoStain.