An Analytical Evaluation of Parasep Stool Concentrators Compared to Conventional Methodologies for Ova & Parasite Fecal Concentration

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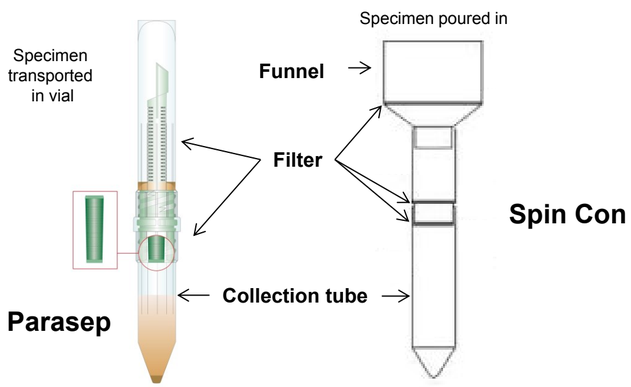
Introduction

Microscropic examination of feces is a standard laboratory method for diagnosing gastrointestinal parasite infections. In North America, the ova and parasite (O&P) examination is typically performed using stool that is chemically fixed in polyvinyl alcohol (PVA) and formalin, after which the stool is concentrated by filtration to enhance sensitivity. Mini Parasep® tubes containing Alcorfix® allow for collection and concentration within a single collection vial. These tubes were evaluated analytically and clinically compared to the standard PVA/formalin collection and processing method which use a SpinCon® concentrator device. In addition, a work-flow analysis was performed showing significant time savings.

Results

Filter Efficiency

Side-by-side comparisons between two concentrator devices were performed with 47 positive ova & parasite exams submitted to ARUP Laboratories during July and August 2014. An equal volume of fixed stool (3mL) was added to the SpinCon® filter and the Mini Parasep® tubes for equal specimen volume comparison. The sediments from both concentration methods were then analyzed by trichrome stain (permanent slide) and wet mount preparation with Lugul's iodine for contrast. Identification along with an examination of organism morphology and obstruction from background debris was documented. Filter efficiency between the two stool concentrator devices was comparable with only one discrepant specimen. The discrepant specimen was positive for *B. hominis* and *Entamoeba coli* by Parasep® concentration but only positive for *B. hominis* by SpinCon®.



Results

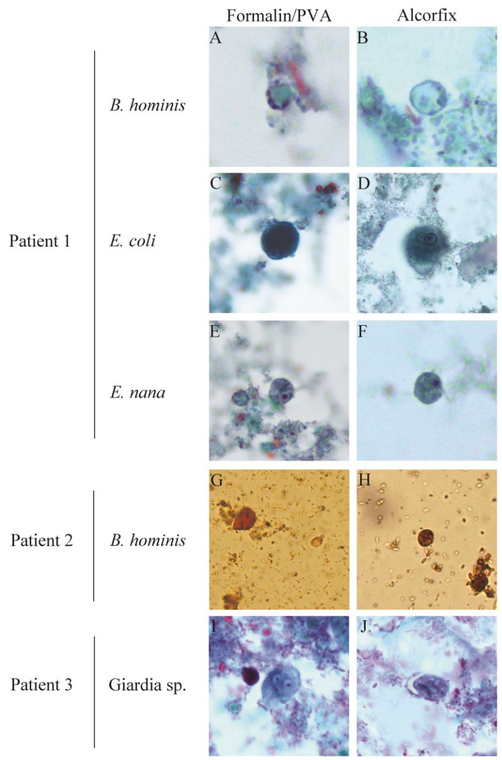


Figure 1: Positive stool specimens co-collected in PVA/formalin and Alcorfix. Representative protozoa are shown for both fixative types. Trichrome stained images (patient 1 & 3) werecaptured at 1000X magnification with an oil immersion lens and wet mount images (patient 2) were captured at 400X magnification

Clinical Evaluation

The ability of Alcorfix® to preserve morphology and serve as a suitable alternative to PVA and formalin was evaluated in real-time at the University of Utah hospital and clinics. Stool collection kits with the standard PVA /formalin and the Mini Parasep® tube were provided for O&P collection. A total of 26 specimens were submitted for testing in which both fixatives were filled with stool at the required specimen volumes. Three specimens contained parasites of the 26 submissions meeting inclusion criteria (Figure 1). Alcorfix® containing Mini Parasep® tubes showed acceptable performance for conventional trichrome stain and wet mount evaluation; however no coccidian parasites were encountered in our prospective co-collection study.

Results

To test whether Alcorfix® would be compatible with modified acid fast stain and also fix the oocysts such that morphology of the coccidian is retained, live *Cryptosporidium parvum* oocysts were procured and spiked into fresh stool. The stool was separated into vials containing formalin and Alcorfix® and prepared for microscopic examination with a permanent acid-fast stain after concentration in Mini Parasep® tubes. The morphology of the oocysts was maintained in both fixatives (Figure 2A-B). To ensure Alcorfix® with Mini Parasep® tubes can detect microsporidia, live spores were procured, spiked into stool, and fixed in both 10% formalin and Alcorfix® separately. Modified trichrome staining was performed on both fixatives and evaluated microscopically. Both preparations showed conserved morphology predictable for microsporidia, as well as adequate stain retention for both *E. intestinalis* and *E. cuniculi* (Figure 2C-F).

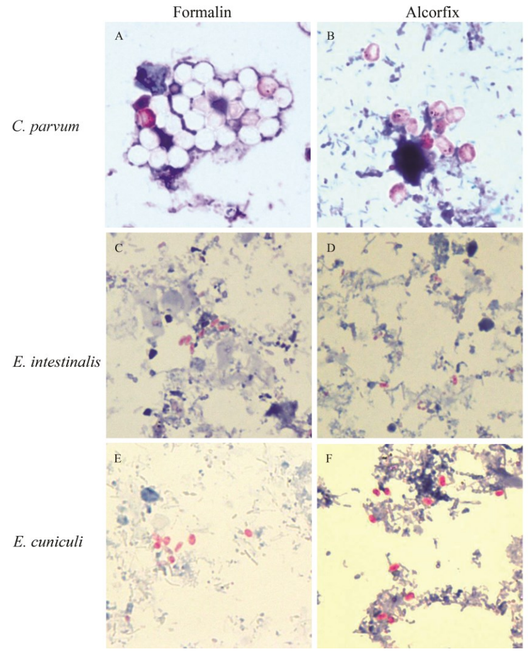


Figure 2: Representative images of stool specimens spiked with live coccidia (*Cryptosporidium parvum,* A-B) or microsporidia (*Encephalitozoon intestinalis* [C-D], *Encephalitozoon cuniculi* [E-F]), fixed in formalin or Alcorfix®, and stained with modified acid-fast stain (A-B) or modified trichrome stain (C-F). All images were captured at 1000X magnification

Results

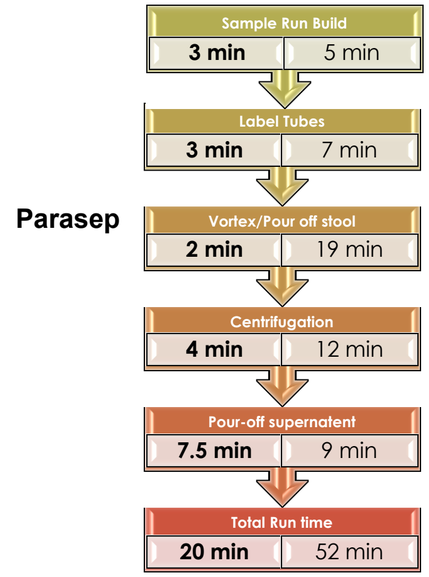


Figure 3: A workflow analysis revealed significant time savings for batches of 30 O&P specimens compared to the same number of specimens submitted in PVA/formalin tubes. Direct hands-on time savings gained by mini Parasep® tubes was 32:01 minutes.

Workflow Study

Three laboratory technicians independently processed batches of 10 or 30 O&Ps prepared in PVA/formalin tubes or Mini Parasep® tubes. Each step was timed and recorded, and the average and standard deviation was calculated for each batch size and concentration method. The average time to prepare a run of 30 O&P specimens was 52 minutes (4:25 standard deviation) using SpinCon® concentration for PVA/formalin, versus 19:45 (standard deviation 1:52) for Mini Parasep®. This difference in workflow represents an average time savings of 32:01.

Conclusions

Mini Parasep® tubes containing Alcorfix® provides a significant workflow advantage to laboratories that process medium to high volumes of O&P specimens. These improvements in workflow, reduction of formalin in the laboratory, and equivalent microscopic examination results are attractive advancements in O&P testing for North American diagnostic parasitology laboratories

Disclosures: Reagents were provided by Apacor and honorarium for conference attendance.