

Evaluation of a Single Vial/Fixative for Parasitic Testing by Microscopy, Antigen Detection, and Real-Time PCR

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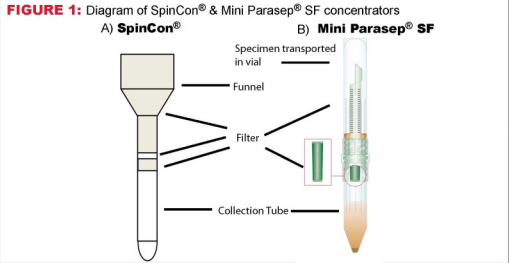
Introduction

Stool collection kits for parasites historically contained three tubes - 10% formalin, PVA (Poly-vinyl Alcohol), and a clean tube. With these three tubes, a variety of tests can be performed: Ova & parasite exams (O&P), specialty stains, antigen detection, and molecular assays. Due to environmental and health hazards related to formalin and mercuric PVA, formalin-free fixatives have been developed that allow parasite detection to be performed from a single vial. We compared our current method of 10% formalin/PVA collection to the use of a single vial mini Parasep® SF tube with either Alcorfix® or SafeFix® (Apacor, Berkshire, UK) for O&P, antigen, and molecular testing.

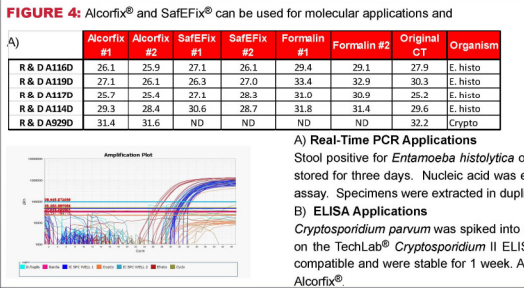
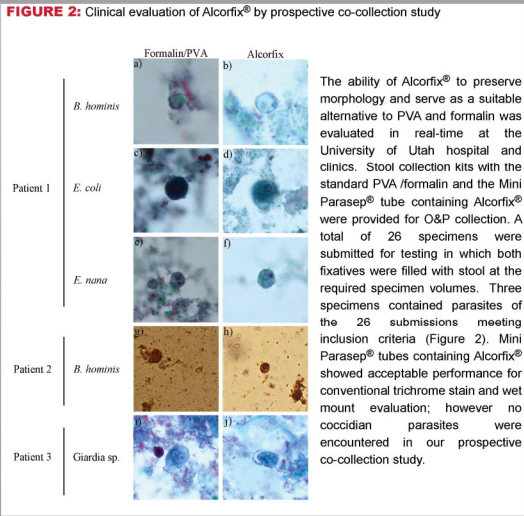
Methods

Spin-Con Methodology with Formalin and PVA:
Stool is submitted to the lab in formalin and PVA vials. Each vial is manually poured-off into a SpinCon® funnel attached to filters and a collection tube (Figure 1A). Tubes are centrifuged for 10 mins. The supernatant is poured-off leaving a pellet which is then used to make either Trichrome (PVA) or Wet-mount (Formalin) slides. Alternatively, Modified acid-fast or modified trichrome stains can be made from the formalin pellet.

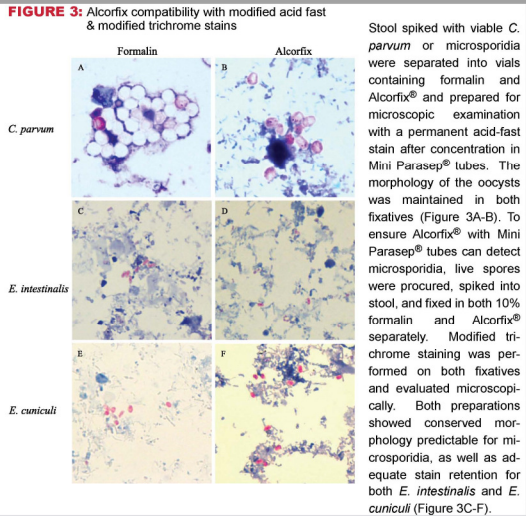
Parasep Methodology with Alcorfix®:
The device is provided to the patient as two separate components which are assembled after collection/inoculation for subsequent transport and processing (Figure 1B). The fixative is contained in a flat-bottom tube containing a screw-off cap, while the vertical filtration device is attached to the conical collection tube assembly. Patients collect two level spoons of stool which are added to the Alcorfix®, or SafeFix®-containing portion of the tube. Alcorfix® is an alcohol-based fixative (ethanol, PVA, isopropanol, methyl alcohol, acetic acid, glycerin, and zinc sulfate) and SafeFix® is a proprietary fixative that is environmentally friendly and alcohol free. Tubes received in the lab are briefly mixed and centrifuged at 400 xg for 2 mins. The supernatant is poured-off leaving a pellet which is then used to make Trichrome and Wet-mount slides.



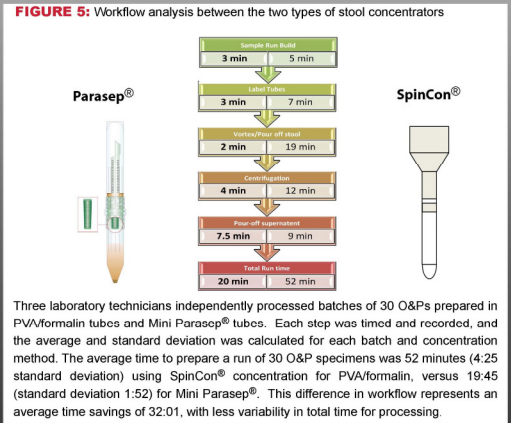
Results



Results



Results



Conclusions

- An additional comparative study performed in our laboratory using both concentration devices on 47 known-positive stool samples revealed:
 - Overall equivalent performance between both concentration methods
 - One discrepant result: *B. hominis* and *Entamoeba coli* detected by Parasep® concentration whereas SpinCon® only allowed detection of *B. hominis*.
- Prospective co-collection shows performance of Alcorfix® is equivalent to PVA/formalin for trichrome stain and wetmount preparations
- Spiking of stool with *C. parvum* or microsporidia and fixing with Alcorfix® or Formalin showed equal or better performance for Alcorfix®
 - SafeFix® also shows equal performance with *C. parvum* (DNS) staining (Microsporidia studies will be conducted in the future)
- Alcorfix and SafeFix® are compatible for molecular applications and ELISA assays
 - Alcorfix may show a slight decrease in antigen stability past 1 week; further studies are needed to confirm this observation
- The single-vial Parasep® concentrator along with Alcorfix® was superior in a workflow analysis saving over 30 mins of time per 30 sample run.

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