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

Brianne Couturier, Jensen R, Arias N, Micheal Heffron, Elyse Gubler, Kristin Case, Jason Gowans, and Marc Roger Couturier

ARUP Laboratories, Institute for Experimental and Clinical Pathology, Salt Lake City, UT;
University of Utah School of Medicine, Salt Lake City, UT

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. Ova to environmental and health hazards related to formalin and mordants. 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 AlorFix® or StatIFx® (Agencourt, Beckman, 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 Spin-Con® funnel attached to filters and a collection tube (Figure 1A). 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 AlorFix®:

The device is processed to the patient as two separate components which are assembled after collection/processing for subsequent transport and processing (Figure 1B). The fixative is contained in a flat-bottomed tube containing a screw-off cap, while the vertical filtration device is attached to the conical collection tube assembly. Patient collects two level spoonfuls of stool which are added to the AlorFix®, or StatIFx® containing portion of the tube. AlorFix® is an alcohol-based fixative (ethanol, PVA, xylene, methyl alcohol, acetic acid, glycerin, and zinc sulfate) and StatIFx® is a proprietary fixative that is ethanol-enormously friendly and alcohol-free. Tubes received in the lab are briefly mixed and centrifuged at 400 x g for 2 min. The supernatant is poured-off leaving a pellet which is then used to make Trichrome and Wet-mount slides.

Results

FIGURE 2: Clinical evaluation of AlorFix® by prospective co-collection study

Stool samples were separated into two groups: one containing AlorFix® and the other containing 10% formalin/PVA. The ability of AlorFix® 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 clinic. Two collection sets were tested with the relevant PVA formalin and the Mini Parasep® tube containing AlorFix® were provided for 10% formalin/PVA collection. Figure 2A shows the workflow analysis between the two steps of stool concentrations.

FIGURE 3: AlorFix® compatibility with modified acid fast & modified trichrome stains

The results of AlorFix® compatibility with modified acid fast and modified trichrome stains are presented in Figure 3A and 3B. AlorFix® and StatIFx® can be used for molecular applications and ELISA applications.

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 Enterobius coli detected by Parasep® concentration whereas StatIFx® only detected the latter.
- Prospective co-collection shows performance of AlorFix® is equivalent to PVA/formalin for trichrome stain and stool-concentrations.

- Spiking of stool with C. parvum or microsporidia and fixing with AlorFix® or Formalin showed equal or better performance for AlorFix®:
  - StatIFx® also showed equal performance with C. parvum (DNS) staining (Microsporidia studies will be conducted in the future).
- AlorFix® and StatIFx® are compatible for molecular applications and ELISA assays.
- AlorFix® may show a slight increase in antigen stability past 1 week; further studies are needed to confirm this observation.

- The single-vial Parasep® Concentration along with AlorFix® was superior at a work flow analysis saving over 30 min of time per 30 sample run.

Contact: marc.couturier@aruplab.com
Poster Board 139