Preparation of Diverse Animal Fecal Specimens with the 2A Automatic Homogenization Processor

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Abstract

Preparing solid excrement for O&P testing is time consuming and requires substantial hands-on analyst time. Herein we present an alternative, automated fecal processing platform, the Sciendox 2A, originally developed for preparing human specimens. The 2A can reproducibly process a sample in less than 2 min. Fecal samples from 10 different animals were successfully processed and compared.

Introduction

Traditional fecal processing methods for parasitological (O&P), oncological and gastrointestinal disease purposes are labor intensive (multiple steps requiring analyst intervention such as mixing and centrifugation, waiting for floatation), susceptible to analyst variability and long (> 10 min).[1,2] The present work introduces our re-development of the Sciendox 2A Automatic Homogenization Processor to prepare animal feces. This platform comprises consumables and a software-controlled, random access sample handling instrument capable of continuously processing up to 60 samples. Fecal samples are converted into a well-suspended filtrate within 2 min for downstream applications including floatation/microscopic analysis, molecular assays and immunoassays [for example, the *Giardia* Chemiluminescence Assay (GCA)]. The steps include automated liquid addition to feces in the sample container, rapid rotational mixing to disrupt and suspend solid material, and double filtration through the bottom of the sample container into a secondary tube that may be used for subsequent tests. This is a closed system that minimizes cross-contamination and analyst contact with feces. The utility of the 2A is demonstrated for processing 10 different domestic animal fecal samples and recovering micron sized particles as surrogates for parasites.

Materials & Methods

Fresh fecal specimens were collected from various sources, with permission. Diet and health status were not monitored, but none of the animals appeared or were reported to be sick. Approximately 0.5 g of each fresh-frozen specimen was collected with a sampling spoon (about the volume of 2 – 3 small green peas) and placed in a sample container. As a processing quality tracer surrogate for parasites, 4×10^5 polystyrene particles (10 µm dia., blue; Magsphere, Inc.) in 0.01% Triton X-100 were added to each specimen aliquot prior to processing. Following software instructions, the 2A automatically recorded barcodes and images of the specimens, added 4 mL of a buffered diluent, suspended the specimens with an animal-specific number of mixing cycles and filtered the < 250 µm fraction into clean test tubes [3,4,5]. Images of the processed sample containers with retentate and test tubes with filtrate were also recorded. Particles in the filtrates were viewed by brightfield microscopy (Revolve, Echo Laboratories) under 10x magnification (Olympus), and the particle numbers were compared to those from a “no specimen” control condition. Results shown are representative qualitative or mean quantitative values from 3 tests.

Results & Discussion

The 2A Automatic Homogenization Processor for diluting, solubilizing and filtering human fecal specimens was re-developed for animal specimen purposes. The 2A platform comprises a:

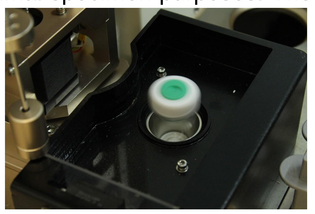
sampling spoon

sample container

close-up of tearing cross

test tube

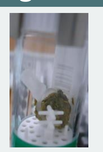
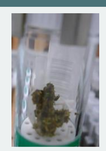
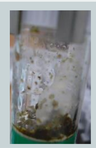
processing deck



close-up of rotational mixing

A reproducible amount of fecal specimen is collected with a sampling spoon, the spoon with specimen are placed in a sample container, the container is closed and added to the processing deck. A robotic clamp automatically picks up the sample container, transports it to a barcode reading position, then to an imaging position and finally to a mixing station. The sample container has a septum on top through which a diluent solution is added. Meanwhile, the robotic clamp picks up a test tube, transports it to the barcode reading position, then to a conveyer beneath the deck. The sample container containing specimen is rotationally mixed, suspending the specimen by a combination of the mixing action and contact with a “tearing cross.” After mixing, the sample container is held in place while the test tube is pressed upward, forcing the homogenate through a pair of filters in the bottom of the sample container while retaining large fecal debris, then through a resealable puncture hole in the cap and into the test tube. The robotic clamp returns the sample container and test tube to their positions on the deck.

Fecal specimens of typical form from 10 domestic animals were processed with the 2A and compared. The filtrates and retentates reflect different animal diets and digestion processes. The number of mixing cycles was adjusted until each animal’s fecal specimen was fully suspended. Most specimens suspended easily from the default 6 mixing cycles or a little more (8 mixing cycles). Sheep and cat feces, however, were particularly cohesive and sticky, making them difficult to process; they required manual removal of the specimen from the sampling spoon and using 12 mixing cycles to disrupt and suspend. All filtrates were fine, opaque green-to-brown suspensions, and replicate filtrates were substantially similar to each other. Filtrates from at least dogs were appropriate matrices for detecting *Giardia* antigens using the GCA (data not shown). Recoveries of the particle surrogates for parasites were generally above about 60%. Notable exceptions to this finding include results from chicken and rabbit feces; determining the reason for the lower recovery from these animals is underway. Fecal specimen testing from additional domestic and exotic animals is ongoing and will be presented elsewhere.

	cow	horse	donkey	sheep	goat	pig	chicken	rabbit	dog	cat
specimen before										
specimen after										
filtrate										
mixing cycles	6	6	6	12	8	8	8	8	8	12
particle recovery	69%	89%	92%	74%	87%	86%	57%	47%	78%	66%

Conclusions: The 2A rapidly processed feces from 10 varied animals into filtrates with minimal analyst intervention. Particles of similar size to many fecal parasites were recovered in high numbers from the filtrates, supporting the 2A as an automated fecal specimen preparation platform for O&P.

References

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