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Fecal collection, ambient preservation, and DNA extraction for PCR amplification of bacterial and human markers from human feces

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Abstract

Feces contain intestinal bacteria and exfoliated epithelial cells that may provide useful information concerning gastrointestinal tract health. Intestinal bacteria that synthesize or metabolize potential carcinogens and produce anti-tumorigenic products may have relevance to colorectal cancer, the second most common cause of cancer deaths in the USA. To facilitate epidemiological studies relating bacterial and epithelial cell DNA and RNA markers, preservative/extraction methods suitable for self-collection and shipping of fecal samples at room temperature were tested. Purification and PCR amplification of fecal DNA were compared after preservation of stool samples in RNAlater (R) or Paxgene (P), or after drying over silica gel (S) or on Whatman FTA cards (W). Comparisons were made to samples frozen in liquid nitrogen (N2). DNA purification methods included Whatman (accompanying FTA cards), Mo-Bio Fecal (MB), Qiagen Stool (QS), and others. Extraction methods were compared for amount of DNA extracted, DNA amplifiable in a real-time SYBR-Green quantitative PCR format, and the presence of PCR inhibitors. DNA can be extracted after room temperature storage for five days from W, R, S and P, and from N2 frozen samples. High amounts of total DNA and PCR-amplifiable *Bacteroides* spp. DNA (34%±9% of total DNA) with relatively little PCR inhibition were especially obtained with QS extraction applied to R preserved samples (method QS-R). DNA for human reduced folate carrier (SLC19A1) genomic sequence was also detected in 90% of the QS-R extracts. Thus, fecal DNA is well preserved by methods suitable for self-collection that may be useful in future molecular epidemiological studies of intestinal bacteria and human cancer markers.

Keywords: *Bacteroides*; DNA extraction; DNA preservation; Enteric bacteria; Feces; Stool

1. Introduction

Feces contain intestinal bacteria and exfoliated epithelial cells that may provide useful information concerning gastrointestinal tract health. For example, bacteria activate or metabolize potential carcinogens (Blaut et al., 2006; Knasmuller et al., 2001; Vanhaecke et al., 2006) or can have anti-tumor effects (Fukui et al., 2001) that may have relevance to colorectal cancer, the second most common cause of cancer deaths in the USA. With the gastrointestinal tract being the largest area of the body that is constantly exposed to ingested/digested food and microorganisms, it is conceivable that luminal exposure may play a significant role in the development of colorectal cancer.

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Epithelial cells in feces represent a potential source of early biomarkers of gastrointestinal tract cancers. Although a variety of biomarkers have been utilized in epidemiological studies on colorectal cancer, most previous markers have been blood-based. However, markers analyzed from intestinal samples may be more relevant to the onset and detection of colon cancer. While approximately 55% of dry fecal weight is attributed to bacteria, Nair and co-workers report that approximately 1.5 million colonic epithelial cells can also be isolated per gram of stool (Desilets et al., 1999; Iyengar et al., 1991). Thus, exfoliated gastrointestinal tract cells in feces may be an alternative for evaluating colon cancer biomarkers.

Stool sample analysis offers a non-invasive opportunity to evaluate both luminal exposure to different types of bacteria as well as exfoliated epithelial cell markers for colorectal cancer risk. However, one of the major obstacles to introducing fecal markers in population studies has been the difficulty in collecting adequate samples for assays from a large number of subjects. This difficulty is exacerbated by the fact that standard fecal collection procedures require fresh or frozen samples, which limits its application in a community-based setting. As a result, epidemiological studies utilizing fecal specimens have often been limited in the number of study subjects and in controlling potential confounders. Fecal self-collection kits have recently been used in large-scale epidemiological studies involving the diagnosis of food-borne illnesses, but these kits lacked any DNA/RNA preservation method, potentially limiting their full usefulness (Jones et al., 2004). Since new technologies have become available to preserve tissue DNA and RNA for a period of time at room temperature, application of such technologies to fecal samples may have great potential for epidemiological studies.

In the present feasibility study, multiple methods for fecal preservation and DNA extraction were tested. Since a major problem with complex samples such as feces is the presence of PCR inhibitors, analytical methods were designed to detect, quantify, and identify conditions under which PCR inhibition was minimal. While this paper focuses on DNA preservation, extraction, and quality, the methods studied were also chosen for their likely suitability for preserving RNA as well. Altogether, several ambient temperature preservation and extraction combinations were capable of yielding usable DNA; however, one combination of ambient preservation and extraction methods gave the most consistent yield of relatively inhibitor-free DNA.

2. Materials and methods

2.1. Stool samples

Fifteen fresh stool samples, obtained from patients being evaluated at the vascular clinic of the John D. Dingell VA Medical Center (Detroit, MI), were collected in plastic containers that were immediately put on ice. The vascular clinic was used for recruitment as it would not be expected that such patients would be more likely than the general population to have colonic abnormalities, as might be the case for a general surgery clinic. This research protocol was approved by the Wayne State University and VA Medical Center Human Investigation Committees and written informed consent was obtained from each study participant. Samples were further processed or transferred to preservative (see below) within 1 h. Although only ten stool samples were needed, fifteen were collected since five samples were inadequate for further processing due to poor consistency (i.e., too watery) or inadequate quantity and were not used in the study. In addition to the above samples collected at the VA Medical Center (referred to, collectively, in this paper as "VA Samples"), preliminary tests of various methods (prior to the above 15 samples) were conducted with anonymously provided stool samples collected by the Ram laboratory, by methods approved by the Wayne State University Human Investigation Committee.

2.2. Sample preparation, preservation, and storage

For each VA sample, 0.2 g aliquots (at least five for each preservative method) were removed by taking cores of the stool sample with a cut-off 1 ml syringe, where 0.2 ml is ≈0.2 g. Each 0.2 g core received one of the preservative treatments, which included spreading and drying on a Whatman FTA card (W; Whatman, Florham Park, NJ.), drying over silica gel beads (S), submersion in 1.0 ml *RNAlater*™ (R; Ambion, Austin, TX.), immersion in 1.0 ml *Paxgene*™ (P; PreAnalytiX, Hombrechtikon, Switzerland), and refrigerator storage (F). Except as noted for pilot tests, the W, S, R, and P preservation methods incorporated a five-day "hold" period at ambient temperature to mimic the likely delay between self-collection of a sample and receipt by an analytical laboratory, for comparison to alternative storage procedures utilizing 24 h refrigeration or immediate freezing in liquid nitrogen.

For W samples, the 0.2 g of feces was spread over two of the four quadrants of the FTA card, allowed to dry approximately 2 h at room temperature, and then placed in a protective barrier pouch with silica gel desiccant packet. For S samples, 0.2 g of feces was placed over silica gel beads (~10 ml) and ~1 cm of glass wool in a 50 ml tightly sealed sterile polypropylene tube. R and P samples were stored in 2 ml sterile polypropylene tubes. After five days storage at room temperature, W and S samples were transferred to −80 °C. Also, after five days, R and P samples were centrifuged (2 min at 10,000 × g), the supernatant was removed, and the pellet was stored at −80 °C. For F samples, 0.2 g of feces was sealed in a sterile 50 ml polypropylene tube and placed in a 4 °C refrigerator for 24 h and then transferred to −80 °C. On the day of collection, remaining portions of each stool sample (designated N2) were placed in paper-lined aluminum foil wrappers, flash-frozen in liquid N_2, and immediately stored at −80 °C. The above methods, along with their associated extraction methods (next section) are summarized in Table 1.

2.3. Sample extraction

DNA extraction procedures included Mo-Bio Fecal (MB; Mo-Bio, Carlsbad, CA.), Qiagen QIAamp DNA Stool Mini