INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic gastrointestinal disorder with increased incidence [1]. The onset of the disease is mainly associated with the immune status, gut microbiota and food intake. The current scientific knowledge of specific triggers and diagnostic markers is limited; therefore, new data on gut microbiota, inflammatory, endocrine and nutritional status are required to better understand the IBD pathophysiology and ultimately support stratification of patients and initiation of appropriate therapy [2,3].

In the translational project Assessment of Microbiota, Inflammatory Markers, Nutritional and Endocrinological Status in IBD Patients (Acronym: MINUTE for IBD) we are exploring host-gut microbiota interactions in order to define novel strategies for the management of IBD. Biological samples (stool, intestine biopsies, blood) as well as food and quality of life questionnaires from 40 newly diagnosed IBD patients and 20 non-IBD control individuals will be collected and analysed in the period 2014-2018 (Fig. 1).

AIM

One of the aims of MINUTE for IBD project is to optimize sensitive methodology of microbiota composition determination via next generation sequencing. Here we present evaluation of different DNA extraction kits and OMNIgene.GUT system as part of the protocol for collection, storage and analysis of microbial composition in human faecal samples.

MATERIALS & METHODS

We compared two different approaches for faeces collection and storage and three commercially available DNA extraction kits. Faeces from 6 healthy donors were collected fresh and in OMNIgene.GUT system, then stored for 14 days at -20°C and at room temperature, respectively. Three extraction methods (MO BID Power fecal DNA isolation kit, QIAamp Fast DNA Stool Mini Kit and MP Biomedicals Fast DNA spin kit for faeces) were evaluated according to DNA yield, quality, integrity, and microbial community structure. DNA yield and quality was measured using Nanodrop and Qubit, followed by Illumina MiSeq sequencing and taxonomic analysis based on V3 and V4 regions of 16S rRNA gene using QIIME [4].

RESULTS

As DNA isolation from complex biological samples is a major step in obtaining high quality DNA, as the first step, the utility of commercially available systems (OMNIgene.GUT stool collection system, and DNA extraction kits: MP Biomedicals, QIAGEN, MO BID) was assessed in healthy volunteers.

It has been demonstrated that DNA yield and quality varied between DNA extraction kits (MP Biomedicals>QIAGEN>MO BID) (Fig. 2 and 3), as well as efficiency of genera extraction (Fig. 4).

Donor-specific bacterial diversity was maintained irrespective of the collection, storage or extraction method used (Component 2 and Component 3 in Fig. 5). Kit-specific patterns were observed by principal component analysis (Component 4 in Fig. 6). More specifically, significantly different levels of abundance among the most prevalent families, notably Bacteroidaceae being increased and Lachnospiraceae decreased when using MO BID kit (Fig. 7). In general OMNIgene.GUT preserves microbiota profile of the most abundant families.

CONCLUSION

The results showed that commercially available systems OMNIgene.GUT and MP Biomedicals are optimal tools for the assessment of human faecal microbiota composition and are being used in the MINUTE for IBD project due to their high convenience and reliability.