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Preliminary Study for Comparison of Commercial Kits for Isolation of *Blastocystis* sp. DNA in Stool Samples from Patients

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Abstract

In molecular diagnosis, the detection of protozoa in fecal samples is hampered by poor recovery of DNA and the presence of PCR inhibitors. In this regard, a reliable extraction of DNA is a key step in the molecular diagnosis of parasitic infections. Currently, there are many commercially available kits for isolation of DNA from feces. Each of these includes a method for removing PCR inhibitors from samples. The purpose of the study was to determine a suitable kit for isolating *Blastocystis* sp. DNA from fecal samples and then use it in PCR to detect *Blastocystis* sp. in the samples.

Three commercially available kits were used to isolate the DNA of *Blastocystis* sp. using human feces samples. The study included stool samples of patients referred for routine laboratory testing for parasites. The samples in which *Blastocystis* sp. was detected by microscopic examination (a native lugol preparation and staining with trichrome) and cultivation in Jones' medium containing 10% horse serum were utilized. QIAamp DNA Stool Mini kit (Qiagen, Germany), ZR Fecal DNA kit (Zymo Research, USA) and Exgene™ Stool DNA mini (GeneAll, Korea) were used for the extraction of *Blastocystis* DNA. The quality and purity of total genomic DNA was measured with a spectrophotometer. Specific primers were employed for the amplification of *Blastocystis* sp. DNA.

In this study, it was observed that Exgene™ Stool DNA mini could not detect *Blastocystis* sp. DNA in gel electrophoresis, while the QIAamp DNA Stool Mini kit and ZR Fecal DNA kit outperformed for isolation of DNA.

Key words: *Blastocystis* sp., stool, DNA extraction, PCR

Резюме

При молекулярната диагностика на протозои във фекалните проби се наблюдават затруднения при получаването на ДНК продукт поради присъствието на инхибитори на PCR реакцията. В тази връзка, подходящата екстракция на ДНК е ключова стъпка при диагностициране на паразитни инфекции. Понастоящем има много налични търговски китове, които изолират ДНК от изпражненията. Всеки от тях включва метод за отстраняване на PCR инхибиторите от пробите.

Целта на изследването е да се определи подходящ кит за изолиране на ДНК от *Blastocystis* sp. от фекални проби и след това да се използва в PCR риакция за откриване на паразита в пробите от това проучване.

Използвани са три търговски кита за изолиране на ДНК от *Blastocystis* sp. от човешки фекалии. Проучването включва проби от изпражнения на пациенти, изпратени за рутинно лабораторно изследване на паразити. *Blastocystis* sp. е установен чрез микроскопски анализ (натурален лигол препарат и оцветяване с трихром) и култивиране в среда на Jones, съдържаща 10% конски серум. За екстракция на ДНК от *Blastocystis* sp. са използвани следните китове: Qiaamp DNA Stool Mini kit (Qiagen, Германия), ZR фекален ДНК комплект (Zymo Research, USA) и ExgeneTM Stool DNA мини кит (GeneAll, корейски). Качеството и чистотата на общата геномна ДНК се измерва със спектрофотометър. За амплификация са използвани специфични праймери.

Получените резултати показват, че при работа с ExgeneTM Stool DNA mini кит в гел електрофорезата не се установява ДНК от *Blastocystis* sp. QIAamp DNA Stool Mini кит и ZR фекален ДНК кит се оказват много подходящи за изолиране на ДНК.

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Introduction

Blastocystis sp. is the most common protozoan in the gastrointestinal system at present. The analysis of fecal Deoxyribonucleic acid (DNA) by nucleic acid methods, in particular, has led to significant advances in the diagnosis and research of *Blastocystis* sp. over the past few years, which allowed the precise identification of carriers and molecular characteristics with high discrimination ability. However, fecal matter is problematic for molecular analysis due to the presence of organic substances. In addition, the presence of PCR inhibitors can cause additional problems for DNA extraction, quantification and amplification.

At present, there are many commercially available kits for isolation of DNA from feces. Although the efficacy of these kits has been tested on different materials, the effectiveness and ability of various commercial kits to extract high-quality DNA from stool specimens has yet to be investigated.

The aim of this study was to compare the effectiveness of commercial DNA extraction kits in extracting clean, high-quality *Blastocystis* sp. DNA from stool samples. In this study, three commercial kits were tested for direct elution of DNA from the same samples containing *Blastocystis* sp. positive feces, and then the detectability of the eluted DNA was compared by spectrophotometry and PCR.

Material and Methods

This study was conducted with the approval of the Committee for the Ethics of Clinical Studies at Gazi University (14.03.2016-135).

Three commercially available kits were used for the isolation of the DNA of *Blastocystis* sp. using human stool samples. The study included the stool samples of patients referred for routine laboratory testing for parasites. The samples detected positive for *Blastocystis* sp. by microscopic examination (a native lugol preparation and staining with trichrome) and cultivation in Jones' medium containing 10% horse serum, were collected. All positive samples were frozen at -20°C until DNA extraction (Yoshikawa *et al.* 2011).

Five samples out of them were selected randomly for DNA isolation through the aforementioned kits (Table 1) according to the manufacturer's protocol. After the extraction, the final DNA yield was quantified using a Nanodrop 1000 spectrophotometer (Thermo Scientific), and the purity of the DNA was determined by the value of OD260/OD280.

The samples were verified for the presence of *Blastocystis* sp. using PCR amplification of *Blastocystis*-specific SSU rRNA using the primers RD5 (5'-TCTGGTTGATCCTGCCAGT-3') and BhRDr (5'-GAGCTTTTTAACTGCAACAACG-3') as recommended previously (Stensvold 2013).

A total of 5 μL of each DNA was run on 2% agarose gel with a 50 bp DNA ladder (BioMatik). The gel was stained with ethidium bromide and photographed using the Gene-Snap gel documentation system (Syngene).

Results

The DNA yields, as determined by UV absorbance, were consistently low for the Exgene™ Stool DNA mini kit but were consistently high for the ZR Fecal DNA and QIAamp DNA Stool Mini kits, which provided significantly higher DNA yields for all stools (Fig. 1).

One of the most important requirement for all of the kits was the ability to obtain high-quality DNA free of contaminants, which allows successful amplification of the extracted DNA. The level of contamination can be determined by examining absorbance ratios since DNA has an absorption peak at 260 nm. DNA purity for contamination with residual proteins can be assessed using a ratio of A260/A280, where ratios lower than 1.7 reflects protein contamination and ratios greater than 1.7 reflect pure DNA. All of the three kits were roughly equivalent with respect to A260/A280 ratios (Fig. 2).

Table 1. DNA extraction kits used in this study and results of PCR amplification .

Name of kit	Company	Country	PCR-positive rate (%)
QIAamp DNA Stool Mini kit	Qiagen	Germany	4/5 (80 %)
ZR Fecal DNA kit	Zymo Research	USA	5/5 (100 %)
Exgene™ Stool DNA mini	GeneAll	Korean	0/5 (0 %)

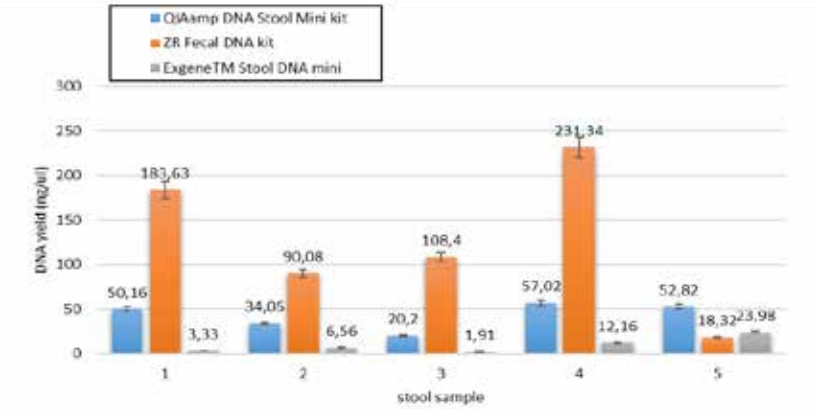

Fig. 1. DNA yields of five stool samples obtained by three commercial DNA extraction kits.

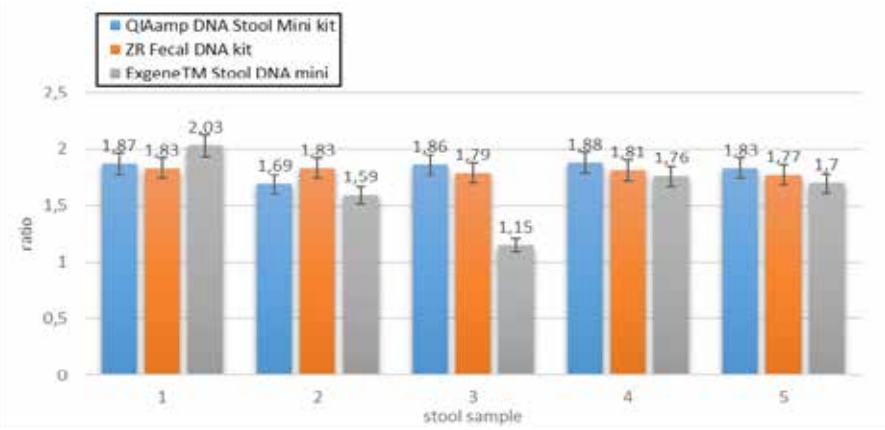

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Fig. 2. Efficiency of commercial kits in removing residual proteins using an absorbance ratio of A_{260}/A_{280} (ratios <1.7 reflect protein contamination, >1.7 pure DNA).

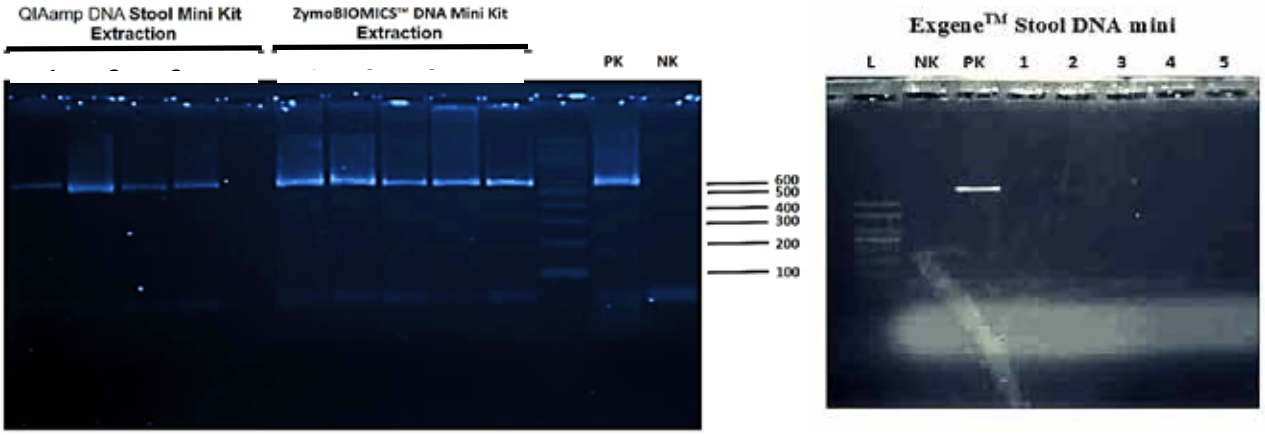

Fig. 3. Agarose gel showing PCR amplification of the same stool sample extracted using three different commercial DNA isolation kits.

Table 2. DNA yields, quality of DNA and PCR amplification results of five stool samples

Stool sample	QIAamp DNA Stool Mini kit			ZR Fecal DNA kit			Exgene™ Stool DNA mini		
	DNA yields	Quality of DNA	Agarose gel showing PCR amplification	DNA yields	Quality of DNA	Agarose gel showing PCR amplification	DNA yields	Quality of DNA	Agarose gel showing PCR amplification
1	++	+++	++	+++	+++	+++	+	+++	-
2	++	++	+++	+++	+++	+++	+	++	-
3	++	+++	++	+++	+++	+++	+	+	-
4	++	+++	++	+++	+++	+++	+	+++	-
5	++	+++	-	+	+++	+++	+	+++	-

+ lowest, ++ average, +++ highest, – unsuccessful

A preliminary test was performed to confirm the utilization of the three commercially available kits for the isolation of *Blastocystis* sp. DNA from fresh fecal samples. The DNA extracted by the two kits purchased from Qiagen and Zymo Research were positive by PCR for partial amplification of the small subunit rRNA gene, while another kit (GeneAll) was negative by PCR amplification in gel electrophoresis, but in the QIAamp DNA Stool Mini kit, one of the five samples was negative, whereas the ZR Fecal DNA kit gave 100% positivity for all samples (Fig. 3).

The ZR Fecal DNA kit provided the highest quality DNA based on successful amplification of *Blastocystis* sp. DNA for all of the five stools. The QIAamp DNA Stool Mini kit extracted high-quality DNA, as demonstrated through successful amplifications of all five stools; however, the amplification of one of the samples in gel electrophoresis was negative (Table 2) (Mahmoudi *et al*., 2011). The DNA extracted from the Exgene™ Stool DNA mini kit was unreliable for PCR and led to some unsuccessful PCR reactions depending on the stool.

Conclusions

In conclusion, the results clearly demonstrate that commercial DNA extraction kits QIAamp DNA Stool Mini and ZR Fecal DNA can be used for the isolation of DNA *Blastocystis* sp. It was also detected that the ZR Fecal DNA kit was the most effective and reliable kit for the isolation of DNA *Blastocystis* sp. since it provided the highest quality DNA that was consistently amplifiable.

Acknowledgements

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