HUMAN *Blastocystis* SUBTYPING WITH SUBTYPE-SPECIFIC PRIMERS DEVELOPED FROM UNIQUE SEQUENCES OF THE SSU rRNA GENE

by

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Thesis Submitted in Partial Fulfillment for the Degree of Bachelor of Medical Laboratory Technology (Hons.), Faculty of Heath Sciences; Universiti Teknologi MARA

2017

AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as reference work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Undergraduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful

Alhamdulillah. Thanks to Allah The Almighty for providing me with good health, blessing and strength in completing this project. I am blessed with all the courage and love given by Him. A thousand of sincere gratitude's goes to my supervisor, Y.M. Dr. Tengku Shahrul Anuar bin Tengku Ahmad Basri, whom I am deeply indebted to. His never ending supports, advices, and critical assessment from the beginning until completion of the study. Thanks also to my supportive co-supervisor, Dr. Mohamad Izwan bin Ismail. I am personally greatfull for having supportive friends, Siti Aisyah Amarang, Aini Suraya Nizam and Nurulain Alias throughout this journey.

Special appreciation dedicated to my beloved parents, Zulkifli bin Abd Majid and Nor Rizan binti Mohamad for all their sincere prayers. The ever ending prayers and support kept me sustained and driven me throughout this study. For those who are not mentioned but had directly and indirectly involved in this study, I am truly appreciate for your efforts and may Allah bless and rewards you. Thank you.

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ABSTRACT

Blastocystis is a single-celled eukaryotic parasite that commonly infects the lower gastrointestinal tract in humans as well as animals. It is genetically diverse whereby 17 subtypes (STs) has been recorded so far. However, ST3 and ST1 are predominant in human stool samples especially in Southeast Asian countries including Malaysia. There is currently a lack of time- and cost-effective detection methods for these STs. Thus, the present study was carried out to develop subtype-specific primers from unique sequences of the SSU rRNA gene of human *Blastocystis* sp. In this study, 2 sets of primers for both ST1 and ST3 have been developed via PerlPrimer and Oligo Analyser software using sequences acquired from the NCBI database. Next, PCR amplification, followed by gel electrophoresis (1.5% agarose gel, 145 volt, 60 minutes) was performed. The sensitivity of the primers were tested using 10-fold dilutions. The primers were also tested against common enteric bacteria and parasites to ensure its specificity. Each primer pairs successfully amplified the target region of the *Blastocystis* samples, whereby 138 bp and 233 bp amplicons were acquired for ST1 and ST3, respectively. Furthermore the detection limits of the multiplex PCR were confirmed at 7 ng/µl for the former, and 70 ng/µl for the latter. The primers were also confirmed to be non-specific to the non-*Blastocystis* samples. Because of the ease of use, specificity and increase in *Blastocystis* infection in Malaysia, this assay has the potential to greatly improve clinical diagnosis of *Blastocystis* infection and becoming method of choice.

Keywords: *Blastocystis*, Human, Primer, Subtype, PCR, Malaysia.