UNIVERSITI TEKNOLOGI MARA

OPTIMIZATION AND EVALUATION OF SARCOCYSTOSIS THERMOSTABLE PCR DETECTION KIT

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced 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 Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Sarcocystis spp. is a parasite that can cause sarcocystosis in human and animals. They are intracellular protozoan parasite that can be found mostly in cattle, sheep and goats. Diagnosis of *Sarcocystis* spp. is mainly carried by histological investigation or serological methods. Histological examination has limitations, as it depends on the site of collection and load of parasitic infection. On the other hand, serological diagnosis of active infection is also unreliable because activation is not always accompanied by changes in antibody levels. Molecular-based techniques are very efficient, as detection of large sample size can be carried out with enhanced specificity and sensitivity. Hence the most reliable method would be PCR technique. However, the conventional PCR requires trained personnel and cold chain transportation/storage. With the knowledge of the disease caused by the organism, its prevalence and geographical distribution, there is a need to develop and improvise the existing molecular tools which can be handy for early detection of *Sarcocystis* in rural areas, where facilities and molecular biologist might not be available, whilst reducing sample collection to processing time. Thermostable premix to detect *Sarcocystis* spp. was developed to make conventional PCR more user-friendly. The aim of this study is to develop a sarcocystosis thermostable PCR detection kit and evaluate this kit in terms of the sensitivity and specificity, stability, limit of detection (LOD), and repeatability. PCR amplification was carried out to detect the D2 region in conserved regions of 18S rRNA in *Sarcocystis* spp. A pair of primers to detect *KMT1* gene in *Pasteurella multocida* was used as internal control. *Sarcocystis capranis* was used as positive control for this kit. The premix contents were lyophilized in the presence of enzyme stabilizer to ensure the *Taq* polymerase work efficiently at room temperature. A total number of 48 samples were used to test the specificity and sensitivity while the stability of this PCR kit was evaluated using Q10 accelerated aging method. The sensitivity and the specificity of the developed thermostable PCR kit was 100%. The Q10 accelerated ageing method at 37°C was found to be about 2.4 month. The limit of detection (LOD) was evaluated using two-fold dilution DNA of *Sarcocystis capranis*. The limit of detection is 0.547 ng/µL. In the repeatability test, different samples with various concentrations of DNA samples were used. The repeatability study was used to observe the consistency of the PCR results from thermostable sarcocystosis premix based on the intensity of the bands. The percentage of coefficient of variance (COV) ranged from 1.00 % to 3.22 %. From this result, it is evident that this thermostable PCR kit has a good range of repeatability. The developed sarcocystosis thermostable PCR detection kit is very efficient in detection of *Sarcocystis* spp. with high sensitivity and specificity with an advantage of being a cold chain free PCR system.

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