

## Abstracts of Theses Approved for the MSc at the Institute for Research in Molecular Medicine (INFORMM) Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia

### DEVELOPMENT OF DNA-BASED DIAGNOSTIC TESTS FOR THE DETECTION OF *SHIGELLA DYSENTERIAE*, *SHIGELLA FLEXNERI*, AND *SHIGELLA SONNEI*

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**Introduction:** Shigellosis or bacillary dysentery caused by *Shigella* spp. remains as significant health problem worldwide. Current detection and identification methods of the pathogens from stool specimens by culture method is relatively insensitive, generally time consuming (approximately 2–5 days) and laborious.

**Objectives:** In this study, thermostabilised polymerase chain reaction (PCR) assays were developed for the detection of *ompA* genes in *Shigella dysenteriae*, *Shigella flexneri*, and *Shigella sonnei*. The tests contained specific primers for the detection of *S. dysenteriae*, *S. flexneri*, and *S. sonnei*, respectively, freeze-dried or thermostabilised PCR reagents with internal control (IC), and gel loading dye.

**Methods:** Primers targeting *ompA* genes for *S. dysenteriae*, *S. flexneri*, and *S. sonnei* were designed. Optimisation of PCR parameters including concentration of IC DNA template, annealing temperature, and MgCl<sub>2</sub> concentrations was performed. In assessing specificity and sensitivity, the primers were challenged with known *S. dysenteriae*, *S. flexneri*, *S. sonnei*, and other enteric pathogens. Thermostabilised PCR-mix periodic stability assessment for each test was done at ambient temperature (25–26 °C) and 4 °C. Optimisation of stool culture in Gram Negative broth (GNB) was done in order to measure optimum incubation time prior to PCR assay. Preliminary evaluation of the developed tests was done using children seeded stool culture in GNB with *S. dysenteriae*, *S. flexneri*, *S. sonnei*, and other enteric bacteria (*n* = 50).

**Results:** None of the DNA template from non-targeted organisms was amplified. After optimisation of IC DNA template concentration was done, 5 pg/μL of IC was incorporated into the assays. Minimum concentration of genomic DNA that could be detected by the assays was 0.39 ng/μL for *S. sonnei* and *S. flexneri*. As for the detection of *S. dysenteriae*, the minimum concentration was 0.78 ng/μL. At bacterial level, the sensitivity was found to be  $4.0 \times 10^3$  CFU/PCR reaction for the test developed for the

detection of *S. dysenteriae*, and  $4.0 \times 10^4$  CFU/PCR reaction for *S. flexneri* and *S. sonnei*. Results suggested that the reagent is stable until 6 months of storage at both temperatures. The optimum time for stool culture in GNB was 8–12 hours for all tests. Preliminary evaluation using seeded stool culture gave 100% for PCR sensitivity and specificity, with 100% positive predictive value (PPV) and negative predictive value (NPV) for the test developed for the detection of *S. dysenteriae* and *S. sonnei*. As for the detection of *S. flexneri*, the sensitivity was found to be 100%, and for specificity, 92.8%. Therefore, the PPV and NPV was 92.8% and 100% respectively.

**Conclusion:** We have developed simple, rapid, sensitive, specific, and cost-effective tests with built-in control for the detection of *S. dysenteriae*, *S. flexneri*, and *S. sonnei*. However, further studies with a larger set of sample, probably using adult stool, need to be done to evaluate the true performance of the developed tests.

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### UTILISATION OF FERMENTATION AND PURIFICATION STRATEGIES TO ENHANCE THE YIELD OF *BmR1* ANTIGEN

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**Introduction:** Brugia Rapid is an established diagnostic test that is commercially available to detect both *Brugia malayi* and *B. timori* infections (brugian filariasis). The test is a dipstick lateral flow test format that utilises *BmR1* recombinant antigen expressed by the clone *Bm17DIII/pPROEXTMHT/TOP 10*. Due to a significant demand for the test in the market, there is a need to scale up the production of the recombinant antigen and increase the purification efficiency to reduce the production cost of the test.

**Objectives:** The objectives of this study were to increase the cell mass of *Bm17DIII/pPROEXTMHT/TOP10* recombinant bacteria and to enhance the expression of *BmR1* recombinant antigen. In addition, the study aimed to improve the purification strategy in order to obtain a high yield of *BmR1* recombinant antigen.

**Methods:** The cultivation of the recombinant bacteria was performed using fed-batch fermentation, where cells were grown in modified Terrific broth medium and glucose and fed exponentially at a controlled rate, using Multifermenter Control Software for automated feeding, during pre-induction and post-induction feeding. Parameters of fed-batch culture, such as the medium composition, growth conditions, feeding, and induction methods, were varied in order to increase the cell mass and produce minimum metabolite inhibition. More specifically, the effects of key fermentation parameters in fermenter, namely pre-induction feeding specific growth rate and the volumetric feed rate of post-induction, were studied. The expression of *BmR1* recombinant antigen was enhanced by varying the protein induction parameters, namely IPTG concentration, frequency, and time point of induction. Efficient purification method was developed in order to increase yield and purity of *BmR1* recombinant antigen by varying the imidazole, salt concentration, and volume of wash buffer used during the affinity chromatography. Lastly, the sensitivity and specificity of *BmR1* recombinant antigen produced in this study were evaluated using ELISA and rapid test.

**Results:** Varying the specific growth rate ( $\mu$ ) prior to induction showed that a final cell concentration of 24.3 g/L was obtained at specific growth rate of 0.15 h<sup>-1</sup> with feeding at high rate during post-induction. However, further increase of the specific growth rate during pre-induction feeding does not produce a higher cell mass; in fact, it decreased the *BmR1* recombinant protein production. It was found that the highest *BmR1* production of 9.82 g/L was obtained when the feeding was carried out at low constant rate (1.9 mL/min), combined with the specific growth rate controlled at 0.075 h<sup>-1</sup>. At this feeding rate, it was also observed that the overall specific productivity, the fermentation yield coefficients, was the highest amongst all the runs tested. This strategy has successfully controlled the accumulation of acetic acid inhibitory by-product below the reported growth inhibitory level of 1.26 g/L. The expression of *BmR1* recombinant antigen was found to be optimal with twice induction of 1 mM of IPTG concentration. Initiation of expression at mid log had generated significant amounts of the soluble protein. Therefore, the *BmR1* recombinant antigen was then purified under non-denaturing conditions using immobilised metal affinity chromatography. In order to increase the efficiency of purification process, various volumes of wash buffer, imidazole, and salt concentration were performed. Imidazole at 20 mM was found to be the best concentration that gave the best yield of *BmR1* recombinant antigen while achieving sufficient purity. When 5 column volumes (CV) of washing buffer was used in the purification step, the production of *BmR1* recombinant antigen was 5.14 mg/L compared with 2.84 mg/L when 10 CV of washing buffer was employed. The overall production of the target protein was improved 7-fold compared with using the conventional flask cultivation method. The sensitivity and specificity of the purified *BmR1* recombinant antigen produced in this study was confirmed by Western blot and 2 other immunoassays techniques, namely

ELISA and immunochromatographic rapid test. In ELISA, it was found that the *BmR1* recombinant antigen produced in this study retained its antigenic property, as it was recognised by sera of filariasis patients (100% sensitive) and not reactive when tested with sera in other infections or from healthy individuals (100% specific). When applied to the rapid dipstick, the *BmR1* recombinant antigen also reacted with the anti-filarial antibodies in the sera of patients' infected with *B. malayi*, with 100% sensitivity.

**Conclusion:** The *BmR1* recombinant antigen produced in this study is useful for the production of Brugia Rapid kit. This study provided an improved method of higher efficiency at a lesser cost for mass-production and downstream processing of *BmR1* recombinant antigen, and this method would be useful for industrial applications.

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