

**EVALUATION OF PUBLISHED LAMP PRIMERS FOR AMPLIFYING
blaCTX-M9 GENE EXPRESSING THE EXTENDED SPECTRUM
BETA LACTAMASE (ESBL) OF *E. COLI***

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**Undergraduate thesis submitted to the faculty of the College of Veterinary
Science and Medicine, Central Luzon State University,
Science City of Muñoz, Nueva Ecija, Philippines
in partial fulfillment of the requirements
for the degree**

DOCTOR OF VETERINARY MEDICINE

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**College of Veterinary Science and Medicine
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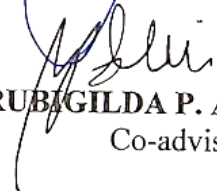
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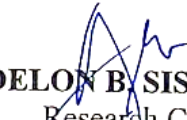
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DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any other university or institution. Information derived from published or unpublished works of others were acknowledged in the text and a list of references was made and included.

Moreover, the undersigned authorizes the Department of Pathobiology of the College of Veterinary Science and Medicine at Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines to make this thesis available for use within the university for the purpose of scholarly research. Except for short citations, which should be properly acknowledged, the information contained therein may not be reproduced in part or in whole without expressed and written permission from the said department.

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ABSTRACT

NOBLE, DOROTHY JOY SALVADOR, Doctor of Veterinary Medicine, College of Veterinary Science and Medicine, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, June 2018, **EVALUATION OF PUBLISHED LAMP PRIMERS FOR AMPLIFYING *blaCTX-M9* EXPRESSING EXTENDED SPECTRUM BETA LACTAMASE (ESBL) OF *E. COLI***

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The study generally aimed to evaluate a Loop-mediated Isothermal Amplification (LAMP) technique in amplifying the gene expressing the ESBL of *E. coli*. Specifically, it aimed to optimize the protocol of LAMP assay for the detection ESBL-producing *Escherichia coli* and to compare diagnostic validity and level of agreement with PCR.

A total of 106 DNA samples were used in the study. For the optimization of LAMP protocol, 12 tubes with ESBL *E. coli* and 12 tubes with nuclease-free water were incubated at a thermal cycler in temperatures ranging from 60-63°C for 30-90 minutes. Validation of results was based on the ability of the positive controls to change in color by adding a SYBR dye, fluorescence under ultraviolet light and the presence of marker in gel electrophoresis upon query. The agreement using Kappa statistics between LAMP and PCR was used to determine the level of agreement of the two methods.

For LAMP assay amplification, amplification of the target gene was observed at 63°C for 60 minutes while the Kappa coefficient was only 0.205, which means that there was a slight agreement between the LAMP and PCR.

Key words: ESBL, *E. coli*, Loop-mediated Isothermal Amplification (LAMP)

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