Cairo University
Faculty of Veterinary Medicine
Department of Virology

Development of multiplex real time PCR assay for detection of some equine respiratory viruses

A Thesis Presented By

Shimaa Mousleh Sayed Ghoniem

(B.V.Sc., Faculty of Veterinary Medicine, Cairo University, 2010)

For the degree of Master in Veterinary Medical Science

(Virology)

Under supervision of

Prof. Dr. Hussien Aly Hussien Ahmed
Professor of Virology
Faculty of Veterinary Medicine
Cairo University

Dr. Ayman Hany El-Deeb
Assistant Professor of Virology
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Mohamed Galal Aggour
Head of Research of Biotechnology
and Ex Director of Animal Health
Research Institute
Agriculture Research Center

(2017)

Approval Sheet

This is to approve that Thesis presented by

Shimaa Mousleh Sayed Ghoniem

For the degree of MV.Sc. (Virology) has been approved by the examining committee

Prof. Dr. Hanafy Mahmoud Madbouly
Professor of Virology
Faculty of Veterinary Medicine
Bani sueif University

Prof. Dr- Mohamed Abd El-Hamid Shalaby
Professor of Virology
Faculty of Veterinary Medicine
Cairo University

Prof. Dr- Mohamed Galal Aggour
Professor of Biotechnology
Animal Health Research Institute
Agriculture Research Center (Supervisor)

Prof. Dr- Ayman Hany El- Deeb
Assistant Professor of Virology
Faculty of Veterinary Medicine
Cairo University (Supervisor)

Prof. Dr- Hussein Aly Hussein
Professor of Virology
Faculty of Veterinary Medicine
Cairo University (Supervisor)

2017

العنوان: كلية الطب البيطرى- الجيزة- مصر
تليفون: 37510309- 3571305
الرمز البريدى: 12211
فاكس: 35725240

Cairo University
Faculty of Veterinary Medicine
Department of Virology

SUPERVISION SHEET

Prof. Dr. Hussien Aly Hussien Ahmed
Professor of Virology
Faculty of Veterinary Medicine
Cairo University

Dr. Ayman Hany El-Deeb
Assistant Professor of Virology
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Mohamed Galal Aggour
Head of Research of Biotechnology and
Ex Director of Animal Health Research Institute
Agriculture Research Center

List of Contents

List of Tables

List of Figures

List of Abbreviation

Amino-LNA	Amino-methylene Locked nucleic acid
ANT3	Adenine nucleotide translocator 3
bDNA assay	Branched DNA assay
BHK	Baby Hamster Kidney
BHQ	Black hole quencher
CAM	Chorioallantoic membrane
cDNA	Complementary Deoxyribonucleic acid
Ct	Cycle threshold
Cy3	Carbocyanin 3
Cy5	Carbocyanin 5
DDW	Deionized double distilled water
DFA	Directigen Flu A
DNA	Deoxyribonucleic acid
dNTPs	Nucleotides
dsDNA	Double stranded deoxyribonucleic acid
DSe	Diagnostic sensitivity
EAdV1	Equine adenovirus 1
EAV	Equine arteritis virus
ECE	Embryonated Chicken Eggs
EDTA	Ethelamine Diamine tetra acetic acid
EHV	Equine herpesvirus
EIV	Equine influenza virus
ELISA	Enzyme-linked immunosorbent assay
ERAV	Equine rhinitis virus A
ERBV	Equine rhinitis virus B
ERV	Equine rhinopneumonitis virus
EtBr	Ethidium bromide
FAM	6-carboxy-Fluorescein
FEN-1	Flap structure specific endonuclease-1
FRET	Fluorescence resonance energy transfer
g	Glycoprotein
HA	Hemagglutinin
HDA	Helicase-dependent amplification
HEX	Hexachloro-6-carboxy-fluorescein
HI	Hemagglutination inhibition

HSV	Herpes simplex virus
ICN	Crude gamma ^{32}ATP
iiPCR	Insulated isothermal polymerase chain reaction
IR	Internal repeat
iiRT-PCR	Insulated isothermal reverse transcription polymerase chain reaction
JOE	2,7-Dimethoxy-4,5-dichloro-6-carboxyfluorescein
LAMP	Loop Mediated Isothermal Amplification
LNA	Locked nucleic acid
LUX	Light Upon eXtention
M	Matrix protein
MGB	Minor groove binder
mRNA	Messenger ribonucleic acid
NA	Neuraminidase
NASBA	Nucleic acid sequence-based amplification
NEP	Nuclear export protein
NP	Nucleo-protein
NS	Non-structural
ORFs	Open reading frames
Oxy-LNA	O-methylene Locked nucleic acid
PA	Polymerase acidic
PB1	Polymerase basic1
PB2	Polymerase basic2
PCR	Polymerase chain reaction
RdRp	RNA dependent RNA polymerase
RCA	Rolling circle amplification
REA	Restriction enzyme analysis
RFLPs	Restriction fragment length polymorphisms
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
ROX	6-carboxy-X-Rhodamin
RPA	Recombinase polymerase amplification
rPCR	Real time polymerase chain reaction
rRT-PCR	Real time reverse transcription polymerase chain reaction
Rsq value	R-squared value
RT-PCR	Reverse transcription polymerase chain reaction
rTth	Recombinant thermostable DNA polymerase

SARS	Severe acute respiratory syndrome
S.equi	Streptococcus equi
SNPs	Single-nucleotide polymorphisms
TAE	Tris acetic acid EDTA
TAMRA	6-carboxy-tetramethyl-rhodamine
TET	Tetrachloro-6-carboxy-fluorescein
Texas Red	Sulforhodamine-101-acid-chloride
Thio-LNA	Smethylene Locked nucleic acid
TK	Thymidine kinase
Tm	Melting temperature
TMA	Transcription mediated amplification
TR	Terminal repeat
U_L	Long unique region
U_S	Short unique region
VDAC-1	Voltage-dependent anion channel 1
VI	Virus isolation
vRNA	Viral ribonucleic acid
vRNPs	Viral ribonucleic protein

Name	**Shimaa Mousleh Sayed Ghoniem**
Date of birth	**1/8/1988**
Place of birth	**Cairo**
Nationality	**Egyptian**
Degree	**B.V.Sc in Veterinary Medical Science – CairoUniversity -2010**
Specification	**Virology**
Title	**Development of multiplex real time PCR assay for detection of some equine respiratory viruses**
Supervisors :	**Prof. Dr. Hussien Aly Hussien Ahmed** **Professor of Virology, Faculty of Veterinary Medicine, Cairo University** **Ass. Prof. Ayman Hany El-Deeb** **Assistant Professor of Virology, Faculty of Veterinary Medicine, Cairo University** **Prof. Dr. Mohamed Galal Aggour** **Head of Research of Biotechnology and Ex Director of Animal Health Research Institute, Agriculture Research Center**

Abstract

Viral respiratory diseases are frequently reported in equine species affecting equine industry and cause huge economic losses. In the present study, we developed a multiplex assay for the simultaneous detection of the main viruses that cause respiratory diseases in equine species, Equine herpesvirus type 1(EHV-1) and 4 (EHV-4) and Equine influenza virus (EIV). The primers and probes amplified only the targeted viruses and there were no inter- assay cross amplifications or non-specific interactions. The multiplex assay efficiencies were 92.5%, 97% and 90% and the monoplex efficiencies were 97.4%, 98.2% and 90.7% for EHV-1, EHV-4 and EIV, respectively. The R square values (Rsq) in both forms were greater than 0.990. The performance of the assay was evaluated by analysing 152 different clinical samples from clinically infected horses. EHV-1 DNA was detected as the single causative agent in 12 samples, EHV-4 DNA in 9 samples and both EHV-1 and EHV-4 were detected in 4 samples. EIV RNA was not detected during this study. The accuracy of the assay was confirmed by comparing these results with those obtained from analysing the same samples using commercial rPCR and rRT- PCR diagnostic kits. This multiplex assay is proven to be a sensitive, specific, accurate and cost- effective method for the detection of the target viruses whether they occur as a single agent or as a part of co-infections.

Key words: Multiplex real- time PCR, Equine, Respiratory diseases.