

Sudan University of Science and Technology

College of Graduate Studies

**Evaluation of RT-PCR for Rapid Detection of Vaccine Strains
Of Rift Valley Fever Virus**

**تقييم تقنية التفاعل التسلسلي التلمري
العكسي للكشف السريع عن فيروسات لقاح
حمي الوادي المتصدع**

**A dissertation Submitted in Partial Fulfillment for the Requirement of
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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال الله تعالى

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا
عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العظيم
سورة البقرة - الآية (32)

Dedication

**I dedicate to my parent
for their love and understanding ...
For my sisters and brothers,
who illuminate my path to success..
To my lovely husband
who taught me
patience and success..**

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ABSTRACT

A single-tube conventional gel-based reverse transcriptase (RT) polymerase chain reaction (RT-PCR) assay, for rapid detection of Rift Valley fever (RVF) virus (RVFV) was developed. The RT-PCR assay was evaluated for detection of vaccine (Smith burn) strain of the virus in cell culture. Two pairs of primers (RV1 and RV2), selected from the medium (M) RNA segment of RVFV, were used as a target for RT-PCR amplification. The outer pair of primers (RV1 and RV2) resulted in amplification of a primary 848 base pair (bp) PCR product. Application of this RT-PCR-based assay to the South African vaccine strains (Smith burn) resulted in direct detection of RVFV RNAs in Vero cell culture.

Sensitivity test has confirmed that this technique could be used to detect 1 picogram from the viral genome in vero cells, this degree of sensitivity could be compared with virus detection through virus isolation.

Amplification products were not detected when the RT-PCR-based assay was applied to RNA from other haemorrhagic fevers viruses including Crimean Congo hemorrhagic fever virus (CCHFV), dengue virus; Yellow fever virus, total nucleic acid extracts from uninfected Vero cells. The RT-PCR provides a rapid, sensitive and specific assay for detection of RVFV in cell culture. The assay could be recommended for inclusion during an outbreak of the disease among susceptible populations.

الخلاصة

في هذه الدراسة أستخدمت تقنية التفاعل التسلسلي التلمري العكسي للكشف عن جينوم فيروس حمي الوادي المتصدع في خلايا كلي القرد الاخضر الافريقي (الفيرو).

أستخدمت عترة فيروس سميث بيرن (جنوب أفريقيا) و المستخدم حاليا كلقاح لهذا المرض كضابط تجريبي حيث تمت عدوي خلايا أحادية الطبقة للحصول علي التأثير الممرض لهذا الفيروس في خلايا الفيرو. تم إستخلاص الحمض النووي الرايبوزي للفيروس من خلايا الفيرو المصابة وذلك بواسطة مستخلصات معملية تجارية. في هذه التقنية أستخدم زوجان من المبدئات تم تصميمهما من الجين الأوسط للفيروس لتكبير جزء من الجينوم بطول 848 زوج قاعدي لفيروس حمي الوادي المتصدع. أثبتت تجارب الحساسية أن هذه التقنية يمكن أن يستفاد منها في الكشف عن 1 بيكو جرام من جينوم الفيروس في خلايا الفيرو وهذه الدرجة من الحساسية يمكن مقارنتها مع تشخيص الفيروس بواسطة عزل الفيروس. أثبتت تجارب الخصوصية أن هذه التقنية يمكنها التمييز بين فيروسات الحمي النزفية الاخرى حيث إن الحمض النووي الرايبوزي المستخلص من فيروسات الحمي النزفية الاخرى مثل حمي الكنغو النزفية, وحمي الضنك لم يتم تكبيره بهذه التقنية إضافة الي أن الحمض النووي المستخلص من خلايا الفيرو وخلايا الدم من غير المصابين لم يتم تكبيره بهذه التقنية المستخدم فيها هذا النوع من التفاعل التسلسلي التلمري العكسي. يستخلص من هذه الدراسة أن هذه التقنية يمكن أن تستخدم في الكشف عن جينوم فيروس حمي الوادي المتصدع في خلايا الفيرو باستهداف مبدئات من الجين الأوسط للفيروس.

LIST OF CONTENT

Topic	Page No
Versa of Holly Quran	I
Dedication	Ii
Acknowledgment	Iii
Abstract	Iv
Abstract (Arabic)	V
List of Content	Vi
List of Figures	Viii
CHAPTER ONE INTRODUCTION	
1. Introduction	1
1.1 Objectives	3
1.1.1 General Objective	3
1.1.2 Specific Objective	3
CHAPTER TWO LITERATURE REVIEW	
2.1 The Virus	4
2.2 The Genome	4
2.2.1 Small (S) Segment	5
2.2.2 Medium (M) Segment	5
2.2.3. Large (L) Segment	6
2.3. Distribution	6
2.4. Transmission	8
2.5. Clinical Feature	9
2.5.1. Mild form of RVF in humans	9
2.5.2. Severe form of RVF in humans	9
2.6. Clinical feature in animals	11
2.7. Population at risk	12
2.8. Laboratory Diagnosis	12
2.8.1. Virus Isolation	12
2.8.2 Cell Culture	13
2.9. Serological techniques	13
2.9.1. Complement Fixation Test (CFT)	14
2.9.2. Hemagglutination Inhibition Test (HI)	14
2.9.3. Indirect Immunofluorescent assay (IFT)	15
2.9.4. Plaque Reduction Neutralization Test (PRNT)	15
2.9.5. Indirect Enzyme-Linked Immunoassay (I-ELISA)	15
2.9.6. Sandwich Enzyme-Linked Immunoassay (sAg-ELISA)	16
2.9.7. Capture Enzyme-Linked Immunosorbent Assay	17
2.10 Molecular Diagnostic Techniques	17
2.10.1 Nucleic Acid Hybridization Assay	17
2.10.2 Polymerase Chain Reaction (PCR)	18
2.10.3. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)	18
2.10.4. Quantitative Real-Time PCR	20
2.10.5. Nested PCR	20

2.10.6. Reverse Transcription-Loop-Mediated Isothermal Amplification Assay (RT-LAMP)	21
2.11. Control and prevention of RVF	22
CHAPTER THREE MATERIALS AND METHODS	
3.1 Maintenance of Vero Cell culture	23
3.2 RVFV propagation.	23
3.3 Extraction of viral nucleic acid from infected cell culture	24
3.4 Primer selection	24
3.5 Reverse transcriptase (RT) Polymerase chain reaction (RT-PCR) Protocol	25
3.6 Buffers and stains	26
3.7 Electrophoresis	26
3.8 Visualization of results	26
CHAPTER FOUR THE RESULTS	
4.1 Virus Isolation	30
4.2 RT-PCR amplification assay	30
4.2.1 Sensitivity of the first round (primary) RT-PCR	30
4.2.3 Specificity	34
CHAPTER FIVE DISCUSSION	
Discussion	36
CHAPTER SIX CONCLUSIONS, RECOMMENDATION	
Conclusion	39
Recommendations	40
REFERENCES	41

List of Plates

Plate No.	Plate title	Page No.
Plate (3-1)	Techne 412-Thermal Cycler	27
Plate (3-2)	Gel electrophoresis apparatus.	28
Plate (3-3)	Gel documentation apparatus.	29
Plate (4-1)	approximately 80% CPE after 3 days post inoculation of RVFV vaccine strain	31
Plate (4-2)	RVFV-free (Non inoculated) Vero cells monolayer as negative control.	32
Plate (4-3)	Sensitivity of the RT-PCR for detection of the primary 848-bp PCR product using the outer pair of primers (RV1 and RV2).	33
Plate (4-4)	Specificity of the PCR for detection of the primary 848 bp PCR product from RVFV.	35