

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 التبليمرى
العكسى للكشف السريع عن فيروسات لقاح
حمى الوادى المتتصدع**

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Submitted by

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بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِيمِ

قال الله تعالى

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلِمْتَنَا 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 التبلمرى العكسي للكشف عن جينوم فيروس حمى الوادى المتصلع في خلايا كلية القرد الأخضر الأفريقي (الفيلو).

أستخدمت عترة فيروس سميث بيرن (جنوب أفريقيا) و المستخدم حالياً كلقالح لهذا المرض كضابط تجربى حيث تمت عدوى خلايا أحادية الطبقة للحصول على التأثير الممرض لهذا الفيروس في خلايا الفيلو. تم إستخلاص الحمض النووي الرايبوزي للفيروس من خلايا الفيلو المصابة وذلك بواسطة مستخلصات معملية تجارية. في هذه التقنية أستخدم زوجان من المبتدئات تم تصميمهما من الجين الأوسط للفيروس لتكبير جزء من الجينوم بطول 848 زوج قاعدي لفيروس حمى الوادى المتصلع. أثبتت تجارب الحساسية أن هذه التقنية يمكن أن يستفاد منها في الكشف عن 1 بيكتو جرام من جينوم الفيروس في خلايا الفيلو وهذه الدرجة من الحساسية يمكن مقارنتها مع تشخيص الفيروس بواسطة عزل الفيروس. أثبتت تجارب الخصوصية أن هذه التقنية يمكنها التمييز بين فيروسات الحمى النزفية الأخرى حيث إن الحمض النووي الرايبوزي المستخلص من فيروسات الحمى النزفية الأخرى مثل حمى الكنغو النزفية، وحمى الصنك لم يتم تكبيره بهذه التقنية إضافة إلى أن الحمض النووي المستخلص من خلايا الفيلو وخلايا الدم من غير المصايبين لم يتم تكبيره بهذه التقنية المستخدم فيها هذا النوع من التفاعل التسلسلي التبلمرى العكسي. يستخلص من هذه الدراسة أن هذه التقنية يمكن أن تستخدم في الكشف عن جينوم فيروس حمى الوادى المتصلع في خلايا الفيلو باستهداف مبتدئات من الجين الأوسط للفيروس.

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