EVALUATION OF THE SOLANA FLU A AND B ASSAY FOR RAPID IDENTIFICATION AND DIFFERENTIATION OF INFLUENZA A VIRUS AND INFLUENZA B VIRUS

BY

RUJIKORN KANLAYANADONKIT

A THESIS SUBMITED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR

THE DEGREE OF MASTER OF SCIENCE

IN BIOMEDICAL SCIENCES

FACULTY OF SCIENCE

GRADUATE SCHOOL, RANGSIT UNIVERSITY

ACADEMIC YEAR 2021

การประเมินชุดน้ำยา SOLANA INFLUENZA A AND B เพื่อตรวจหาและจำแนกเชื้อไวรัส INFLUENZA A และ INFLUENZA B

โดย
รุจิกรณ์ กัลยาณดลกิตติ์

วิทยานิพนธ์ฉบับนี้เป็นส่วนหนึ่งของการศึกษาตาม
หลักสูตรวิทยาศาสตรมหาบัณฑิต สาขาวิชาวิทยาศาสตร์ชีวการแพทย์
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ปีการศึกษา 2564

Thesis entitled

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by

RUJIKORN KANLAYANADONKIT

was submitted in partial fulfillment of the requirements
for the degree of Master of Science in Biomedical Sciences

Rangsit University
Academic Year 2021

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ACKNOWLEDGEMENTS

This research would not have been successful without the kind assistance and extensive support from several mentors. I would like to express my gratitude to my major advisor, Asst. Prof. Dr. Acharawan Thongmee, Department of Medical Science, Faculty of Science, Rangsit University for her excellent advice, guidance, encouragement, generous donation of time for explanation or discussion the problems of my thesis as well as correction the flaws from the experiment until the thesis was written correctly and completely. I would like to express my gratefulness to my co-advisor and thesis committee, Dr. Ekawat Prasomsab, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, for his useful comments and engagement through the learning process of this thesis.

Special thanks also go to the chairperson of thesis committee, Prof. Emeritus Dr. Wasun Chantratita, Head of the Medical Genome Center, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, who gave me the golden opportunity to do this thesis. His guidance and persistent help throughout this thesis are appreciated. My sincere thanks go to Prof. Emeritus Dr. Wasan Chantrathit and Dr. Ekawat Prasomsab who provided me an opportunity to join their team, and who gave me access to the laboratory and research facilities. Without their precious support, it would not be possible to conduct this research.

I would like to express my gratitude to Assoc. Prof. Dr. Wanida Pongsataporn, my thesis examination committee at Rangsit University for her insightful comments and encouragement which incited me to broaden my research from various perspectives.

I would like to thank Z-Medic Co., Ltd. for supporting materials, equipment, and tools for doing this research. My gratefulness goes to Virology Unit, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University for supporting all the samples, tools and equipment for doing this research. In addition, I would like to thank the Faculty of Science, Rangsit University for laboratory and instrument supports.

I would like to thank my parents and my friends for all their love and support throughout this research.

Rujikorn Kanlayanadonkit

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กิตติกรรมประกาศ

งานวิจัยนี้จะไม่ประสบผลสำเร็จหากไม่ได้รับการช่วยเหลือ และการสนับสนุนจากผู้มีพระคุณหลายท่าน ผู้วิจัยขอขอบพระคุณอาจารย์ที่ปรึกษาวิทยานิพนธ์ ผู้ช่วยศาสตราจารย์ ดร.อัจฉราวรรณ ทองมี ภาควิชาวิทยาศาสตร์การแพทย์ คณะวิทยาศาสตร์ มหาวิทยาลัยรังสิต ที่ให้กำลังใจ ให้เวลาในการอธิบาย แก้ไขปัญหาต่างๆ ในระหว่างการทำวิทยานิพนธ์ รวมทั้งให้ความช่วยเหลือในการเขียนและตรวจสอบวิทยานิพนธ์จนสำเร็จลุล่วงเป็นอย่างดี ขอกราบขอบพระคุณอาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วมและกรรมการสอบวิทยานิพนธ์ ดร.เอกวัฒน์ ผสมทรัพย์ ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล ที่ให้ความคิดเห็นที่เป็นประโยชน์และให้การช่วยเหลือในการทำวิทยานิพนธ์นี้

ผู้ขอกราบขอบพระคุณท่านประธานกรรมการสอบวิทยานิพนธ์ ศาสตราจารย์ เกียรติคุณ ดร.วสันต์ จันทราทิตย์ หัวหน้าศูนย์จีโนมทางการแพทย์ คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล ที่ให้คำแนะนำ และความช่วยเหลืออย่างต่อเนื่องจนการทำวิทยานิพนธ์เสร็จสมบูรณ์ ผู้วิจัยขอกราบขอบพระคุณ ศาสตราจารย์ เกียรติคุณ ดร.วสันต์ จันทราทิตย์ และ ดร.เอกวัฒน์ ผสมทรัพย์ เป็นอย่างสูงที่ให้โอกาสผู้วิจัยได้ร่วมงานวิจัยและอนุญาตให้ใช้ห้องปฏิบัติการและสิ่งอำนวยความสะดวกต่างๆ ในการทำวิจัย หากไม่ได้รับการสนับสนุนจากท่านทั้งสองงานวิจัยนี้จะไม่สามารถดำเนินการได้

ขอกราบขอบพระคุณ รองศาสตราจารย์ ดร.วนิดา พงศ์สถาพร กรรมการสอบวิทยานิพนธ์ มหาวิทยาลัยรังสิต ที่ให้ข้อคิดเห็นต่างๆ เกี่ยวข้องกับงานวิจัย ทำให้ได้เปิดประสบการณ์ด้านงานวิจัยในมุมมองต่างๆ รวมทั้งตรวจสอบวิทยานิพนธ์

ขอขอบคุณ บริษัท ซี เม ดิค จำกัด ที่ให้การสนับสนุนวัสดุ อุปกรณ์ และเครื่องมือในการทำวิจัยนี้ ขอขอบคุณหน่วยไวรัสวิทยา ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล ที่สนับสนุนตัวอย่าง เครื่องมือ อุปกรณ์และสถานที่ในการทำงานวิจัยครั้งนี้ นอกจากนี้ ขอขอบคุณคณะวิทยาศาสตร์ มหาวิทยาลัยรังสิต ที่ให้การสนับสนุนห้องปฏิบัติการและอุปกรณ์ในการทำวิจัย

ขอขอบพระคุณครอบครัวและเพื่อนๆ ที่ให้การสนับสนุนตลอดระยะเวลาของการวิจัยนี้

รุจิกรณ์ กัลยาณดลกิตติ์

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Abstract

Influenza is one of the most important respiratory tract infectious diseases. Currently, several laboratory methods are used for the detection of the Influenza virus, such as viral culture, serology, rapid antigen testing, reverse transcription polymerase chain reaction (RT-PCR), immunofluorescence assays, and rapid molecular assays. The reverse transcription polymerase chain reaction (RT-PCR) is used as the reference method for Influenza detection. RT-PCR is an accurate method but quite expensive. Moreover, it takes at least 4 hours to complete the test. Therefore, the Solana Influenza A+B, an isothermal reverse transcriptase-Helicase dependent amplification (RT-HDA) method, is suggested as an Influenza diagnosis method. Solana Influenza A+B is a new method with minimum turnaround time. This study aimed to evaluate the Solana Influenza A+B assay for the detection and differentiation of Influenza A virus and Influenza B virus by determination of accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and limit of detection (LOD).

A total of 260 samples of nasal swab and nasopharyngeal swab from patients with and without Influenza were tested in comparison with the reference method. The results showed that the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of this technique were 100% in all test parameters. In addition, Solana Influenza A+B isothermal amplification method can detect Influenza A virus and Influenza B virus as low as 1,080 copies/µl and 115 copies/µl, respectively.

It was also shown in this study that the Solana Influenza A+B isothermal amplification method did not show any cross-reactivity when testing in other viral infected samples such as human rhinoviruses, respiratory syncytial viruses A, respiratory syncytial viruses B, coronaviruses, and metapneumoviruses.

It is concluded that the Solana Influenza A+B isothermal amplification method shows high sensitivity and specificity and less time-consuming for Influenza A virus and Influenza B virus detection. This enables doctors to plan treatment quickly and effectively to control outbreaks promptly.

(Total 57 pages)

Keywords: Influenza Virus, Solana Influenza A+B Assay, Real Time RT-PCR

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5809212 : รุจิกรณ์ กัลยาณดลกิตติ์
ชื่อวิทยานิพนธ์ : การประเมินชุดน้ำยา Solana Influenza A and B เพื่อตรวจหาและจำแนกเชื้อไวรัส Influenza A และ Influenza B
หลักสูตร : วิทยาศาสตรมหาบัณฑิต สาขาวิชาวิทยาศาสตร์ชีวการแพทย์
อาจารย์ที่ปรึกษา : ผศ. ร.ต.ท. หญิง ดร. อัจฉราวรรณ ทองมี
อาจารย์ที่ปรึกษาร่วม : ดร. เอกวัฒน์ ผสมทรัพย์

บทคัดย่อ

โรคไข้หวัดใหญ่ (Influenza) เป็นหนึ่งในโรคติดเชื้อในระบบทางเดินหายใจที่สำคัญ ปัจจุบันการทดสอบหาเชื้อไวรัสไข้หวัดใหญ่ (Influenza Virus) ในห้องปฏิบัติการสามารถทำได้หลายวิธี เช่น การเพาะเลี้ยงเซลล์ การตรวจทางภูมิคุ้มกัน การทดสอบหาแอนติเจน การทดสอบด้วยปฏิกิริยาลูกโซ่โพลีเมอเรส การทดสอบด้านอิมมูโนฟลูออเรสเซนส์ และการทดสอบด้านชีวโมเลกุล ปฏิกิริยาลูกโซ่โพลีเมอเรส (Reverse Transcription Polymerase Chain Reaction, RT-PCR) เป็นวิธีอ้างอิงสำหรับการตรวจหาเชื้อไข้หวัดใหญ่ (Influenza A Virus และ Influenza Virus) ซึ่งเป็นวิธีที่แม่นยำแต่ใช้เวลานานอย่างน้อย 4 ชั่วโมง และมีราคาสูง Solana Influenza A+B ซึ่งใช้หลักการของ Isothermal Reverse Transcriptase-Helicase Dependent Amplification (RT-HDA) เป็นแนวทางการตรวจวินิจฉัยเชื้อไข้หวัดใหญ่แบบใหม่ที่ใช้เวลาตรวจสั้น จุดมุ่งหมายของการศึกษานี้เพื่อประเมินประสิทธิภาพของชุดตรวจ Solana Influenza A+B ในการตรวจหาและแยกความแตกต่างของชื้อไข้หวัดใหญ่ Influenza A Virus และ Influenza B Virus และเปรียบเทียบกับวิธีมาตรฐาน RT-PCR โดยทดสอบกับตัวอย่าง nasal swab และ Nasopharyngeal Swab ทั้งจากผู้ป่วยที่สงสัยติดเชื้อ Influenza A Virus และ Influenza B Virus จำนวน 260 ตัวอย่าง

ผลการศึกษาพบว่าความไว ความจำเพาะ ความน่าจะเป็นที่ผู้ป่วยเป็นโรค และความน่าจะเป็นที่ผู้ป่วยไม่ได้เป็นโรค และความถูกต้องของเทคนิคนี้เท่ากับ 100% ทุกพารามิเตอร์ที่ทดสอบ นอกจากนี้วิธีของ Solana Influenza A+B Isothermal Amplification สามารถตรวจพบไวรัส Influenza A และ Influenza B ได้ที่เชื้อต่ำสุด คือ 1,080 cp/ml และ 115 cp/ml ตามลำดับ นอกจากนี้พบว่าเมื่อใช้วิธี Solana Influenza A+B Isothermal Amplification ในการตรวจ ไวรัส Influenza A และ Influenza B ในตัวอย่างที่ติดเชื้อไวรัสอื่นๆ เช่น Human Rhinoviruses, Respiratory Syncytial Viruses A, Respiratory Syncytial Viruses B, Coronaviruses และ Metapneumoviruses ผลปรากฏว่าไม่พบปฏิกิริยาข้ามของเชื้อก่อโรคสายพันธุ์อื่นๆ

ผลการวิจัยนี้ สรุปได้ว่า การตรวจวินิจฉัยโรคไข้หวัดใหญ่ด้วย Solana Influenza A+B มีความไวและความจำเพาะสูง ลดระยะเวลาในการตรวจหาเชื้อได้เร็วขึ้น ทำให้แพทย์สามารถวางแผนการรักษาเป็นไปอย่างมีประสิทธิภาพ และ ทันท่วงที

(วิทยานิพนธ์มีจำนวนทั้งสิ้น 57 หน้า)

คำสำคัญ: ไวรัสอินฟลูเอนซา, Solana Influenza A+B, Real Time RT-PCR

ลายมือชื่อนักศึกษา ..... ลายมือชื่ออาจารย์ที่ปรึกษา .....
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ABBREVIATIONS

Abbreviation	Meaning
µl	Microliter
oC	Degrees Celsius
CPE	Cytopathic effect
Da	Dalton
DNA	Deoxy Ribonucleic Acid
dNTPs	Deoxynucleotide triphosphates
H	Hemagglutinin
HA	Hemagglutinin
LOD	Limited of detection
M2	Matrix 2
MDCK	Madin-Darby cells
$Mg2^{+}$	Magnesium
N	Neuraminidase
NA	Neuraminidase
nm	Nanometer
NPV	Negative predictive value
NTC	Negative control
PCR	Polymerase chain reaction
PoCT	Point-of-care testing
PPV	Positive predictive value
PRC	Competitive process control
Real-Time RT- PCR	Real-Time Reverse Transcriptase – PCR
RNA	Ribonucleic acid
RT-HDA	Isothermal Reverse Transcriptase - Helicase-Dependent Amplification
VTM	Viral transport media

CHAPTER 1

INTRODUCTION

1.1 Background of the research problem

Influenza, an acute respiratory tract infection commonly known as "Flu", is one of the most important infectious diseases due to the emergence of a pandemic many times. Influenza has been recognized since the 16th century and spreads rapidly through communities in outbreaks. The clinical features of this infection are sudden high fever, headache, muscle aches, and fatigue. Clinical manifestation of this disease ranges from asymptomatic to fulminant, depending on the characteristics of the virus and the individual host. In addition, the complications of this disease including viral and bacterial pneumonia, acute respiratory distress syndrome, encephalopathy, myocarditis, pericarditis, and myositis can cause high mortality rate especially in very young and elderly people (Lowen, Mubarekai, Steel, & Palese, 2007; Nayak, Hui, & Barman, 2004).

Influenza is caused by influenza viruses type A, B, and C. Influenza viruses are enveloped single-stranded, negative-sense, segmented RNA viruses belonging to the family *Orthomyxoviridae* and are classified into four distinct types; Influenza A virus, Influenza B virus, Influenza C virus, and Influenza D virus (Medina & García-Sastre, 2011; Qi et al., 2011; Taubenberger & Morens, 2010). The four virus types differ in host range and pathogenicity. Influenza A and Influenza B viruses contain eight discrete gene segments, each segment coding for at least one protein. They are covered with projections of three proteins: hemagglutinin (HA), neuraminidase (NA), and matrix 2 (M2). Each influenza RNA segment is encapsidated by nucleoproteins to form ribonucleotide nucleoprotein complexes. Influenza B and Influenza C viruses are isolated almost from humans. Influenza A virus derived from an avian reservoir can infect a wide variety of warm-blooded animals including humans, swine, horses, dogs, cats, and other mammals. Aquatic birds are the natural reservoir for many subtypes of influenza A virus

and probably are the source of human pandemic influenza strains (Taubenberger & Morens, 2010).

Clinical diagnosis of influenza is difficult because symptoms are varied and have common characteristics with other respiratory viruses. Signs and symptoms of Influenza such as fever and cough as well as patient's history and patient's community were usually used for clinical diagnosis. Moreover, the accuracy of clinical diagnosis is influenced by the host's characteristics and the prevalence of influenza in the community. Laboratory investigations for this infectious agent aid in the diagnosis and can be used to guide treatment decisions, avoid inappropriate use of antibiotics, and provide information for influenza surveillance. Laboratory tests for Influenza virus include viral culture, viral antigen detection, and nucleic acid testing (Paules & Subbarao, 2017).

Currently, laboratory diagnosis of Influenza virus can be done using various methods such as viral culture, immunochromatography for viral antigen detection and Real-Time Reverse Transcriptase – PCR (Real-Time RT-PCR) for viral ribonucleic acid detection. Viral culture is a gold standard method and is also used for vaccine production. Immunochromatography is a rapid method but the sensitivity of this method is low. Real-Time RT- PCR provides high accuracy but is expensive and takes about 3-5 hours (Landry, 2011). Detection of Influenza virus by Real-Time RT-PCR shows reliability but it requires multiple diagnostic devices and high equipment provided only in large laboratories. Since the diagnosis of Influenza is important for prompt treatment and epidemic control, a rapid and effective detection method of Influenza virus needs to be developed to reduce morbidity and mortality in viral infected patients.

The Solana Influenza A+B Assay has been developed to address the shortcomings of the current commercial molecular devices while improving upon their benefits. It is used for determination of viral nucleic acids from the sample without the need for extraction. The assay consists of two major steps: 1) specimen preparation and 2) amplification and detection of target sequences specific to influenza A and influenza B using isothermal Reverse Transcriptase - Helicase-Dependent Amplification (RT-HDA) in the presence of target-specific fluorescence

probes. This technology requires a small, integrated device and is less time-consuming. Therefore, the Solana Influenza A+B assay may offer a good alternative rapid and accurate detection of Influenza viral RNA. The objectives of this study are to evaluate the performance of Solana Influenza A+B Assay for the detection of Influenza A virus and Influenza B virus and to compare with the Real-Time RT- PCR reference method.

1.2 Objective of research

To evaluate the Solana Influenza A+B assay for detection and differentiation of Influenza A virus and Influenza B virus by determination of accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and limited of detection (LOD).

1.3 Research Hypothesis

Solana Influenza A+B assay may be high sensitivity and specificity. It can also reduce the time of examination to allow doctors to diagnose patients more quickly. It also helps to reduce the cost of patients.

1.4 Scope of the research

The Influenza test by Solana Influenza A+B assay is a test that distinguishes Influenza from other respiratory tract infections. Viral RNA is extracted from the specimen of patients with suspected Influenza infection with an EASYMAG automatic extractor and then viral genomes are detected with the Solana Influenza A+B assay. The results from the Solana Influenza A+B assay are compared with results from Real-Time PCR as a reference method.

In addition, the sensitivity, specificity, likelihood ratios for a positive test, likelihood ratios for a negative test, positive predictive value (PPV), negative predictive value (NPV), accuracy and limited of detection (LOD) of the Solana Influenza A+B assay for Influenza virus detection will also be determined.

1.5 Benefits of the research

Solana Influenza A+B assay using Isothermal reverse transcriptase-Helicase dependent amplification (RT-HDA) may be used in the detection of Influenza with high sensitivity and specificity. The test results can be realized in 40 minutes compared to Real-Time PCR which takes about 4 hours. These will help doctors diagnose influenza infection faster.

CHAPTER 2

LITERATURE REVIEW

2.1 Influenza Virus

Influenza viruses are single-stranded, negative-sense, segmented RNA viruses belonging to the family *Orthomyxoviridae* and are classified into four distinct types: Influenza A virus, Influenza B virus, Influenza C virus, and Influenza D virus (Medina & García-Sastre, 2011; Qi et al., 2011; Taubenberger & Morens, 2010). Influenza A virus and Influenza B virus can cause outbreaks and seasonal epidemics of disease. Influenza A virus generally causes more severe and widespread disease (Medina & García-Sastre, 2011; Taubenberger & Morens, 2010). Influenza C virus causes a mild respiratory upper tract illness (Matsuzaki et al., 2006; Rosenthal et al., 1998). Influenza D virus primarily affects cattle and is not known to infect or cause illness in people (Collin, et al., 2015; Sreenivasan, et al., 2015).

There are significant differences in genetic material organization, structure, host range, epidemiology, and clinical characteristic among Influenza A virus, Influenza B virus and Influenza C virus (Medina & García-Sastre, 2011; Suarez & Schultz-Cherry, 2000; Uyeki, 2009). However, all three types of Influenza viruses share certain features such as host cell-derived envelope, envelope glycoprotein of virus entry, a segmented genome of negative sense, and single-stranded RNA. Influenza A virus was isolated by Francis from a patient in Puerto Rico in 1934; A/Peuto Rico/8/34 is sometimes referred to as PR8 virus (Francis, 1935, 1937). Influenza A virus was further divided into subtypes based on their hemagglutinin (H or HA) and neuraminidase (N or NA) antigen such as H1N1 or H3N2 (Bouvier & Palese, 2008; Fouchier et al., 2005; Scholtissek, Rohde, Von Hoyningen, & Rott, 1978).

2.2 Structure of Influenza Virus

Influenza viruses are enveloped viruses that may exist in spherical or filamentous forms of 80-120 nm with surface projections consisting of HA and NA spikes (Bouvier & Palese, 2008). The HA spike is the viral attachment protein and the receptor-binding site located in the globular head of the molecule (Bouvier & Palese, 2008). Each rod-shaped HA spike measures approximately 4 nm in diameter by 14 nm in length (Bouvier & Palese, 2008). Each spike is a trimer composed of three HA polypeptide molecules each with a molecular weight of 75,000-80,000 Da resulting in an approximate molecule of around 224,640 Da (Fouchier et al., 2005; Green et al., 1982; Wu & Wilson, 2017). The HA is synthesized as a monomer (HA_0) which is cleaved by host cell protease into HA_1 and HA_2 components that remain linked together. The potential role of HA cleavability in pathogenesis in humans is currently unknown (Green et al., 1982). The viral NA is an enzyme that catalyzes the removal of terminal sialic acid (N-acetyl neuraminic acid) from sialic acid containing glycoprotein (Green et al., 1982). The NA spike is shaped like a mushroom rather than a rod and has a molecular weight of 240,000 Da (Fouchier et al., 2005; Green et al., 1982). The intact NA consists of a tetramer of NA polypeptides, each with molecular weight of 58,000 Da whereas the active site of the enzyme is located in the mushroom shape (Wu & Wilson, 2017). Influenza viruses enter the cell by attachment of the viral HA to the sialic acid-containing receptors on the cell membrane followed by internalization of the virus into the acid endosome (Figure 2.1).

At least 16 highly divergent, antigenically distinct HAs have been described in Influenza A virus (HA1 to HA16) as well as at least 9 distinct NAs (NA1 to NA9) (Wu & Wilson, 2017). Influenza B viruses have a similar structure to Influenza A viruses but they do not exhibit the same type of antigenic and genetic variation in the HA and NA; therefore, they do not have subtypes. However, in 2001, two antigenically distinct lineages of Influenza B viruses called “Victoria” lineage and “Yamagata” lineage have co-circulated in humans (Xu et al., 2004).

The Influenza HA molecule initiates viral infection by binding to receptors on specific host cells and antibodies against the HA protein produced by the host cells may prevent re-infection with the same viral strain by blocking either attachment or cell fusion. Since the

influenza viral RNA polymerase complexes have no proofreading activity, high mutation rates occurred and led to the accumulation of point mutations during replication. Mutations change amino acids in the antigenic portions of surface glycoproteins make viral strains evade preexisting host immunity (Taubenberger & Morens, 2010).

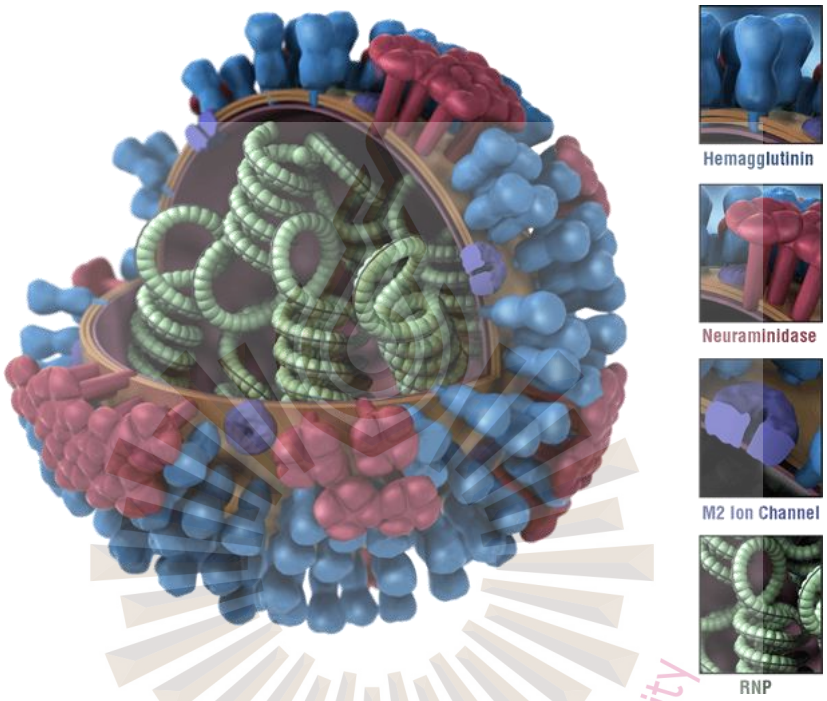


Figure 2.1 A schematic diagram of an influenza virus

Source: Centers for Disease Control and Prevention, 2021

2.3 Epidemiology of Influenza Virus

Influenza viruses are transmitted by the respiratory route and cause large epidemics, which generally occur during winter. In addition, Influenza A viruses can infect a wide variety of animals, particularly migratory waterfowl. New Influenza A virus subtypes sporadically emerge in humans to cause widespread disease or pandemics (Figure 2.2).

Influenza epidemics are regularly associated with excess morbidity and mortality, especially in immunocompromised patients. Influenza virus may lead to pneumonia and acute respiratory distress syndrome that cause high mortality rate in very young and elderly people.

In 1918 pandemic of the H1N1 strain of influenza A virus occurred while the H2N2 pandemic occurred in 1957. In addition, a pandemic of the H3N2 strain of the influenza A virus occurred in 1968. Since then, both influenza A subtypes H3N2 and H1N1 have been co-circulated in humans (Taubenberger & Morens, 2010).

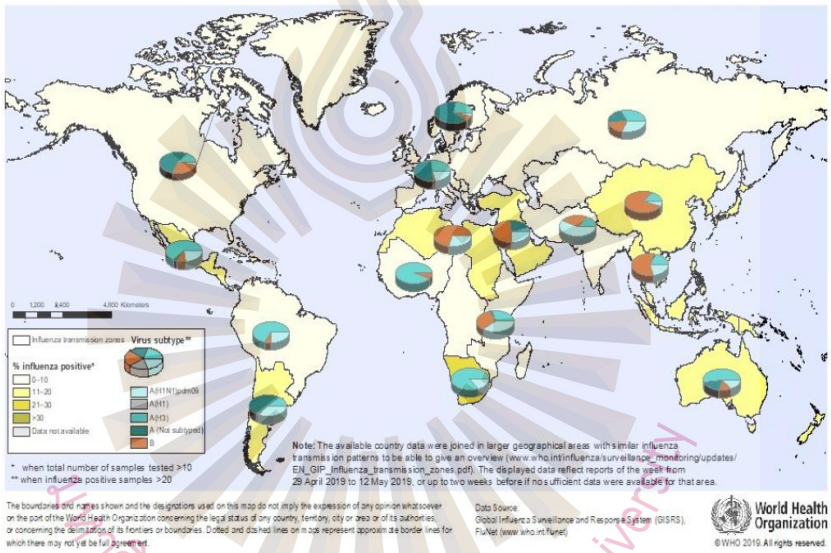


Figure 2.2 Percentage of people infected with Influenza viruses

Source: World Health Organication, 2021

The first Spanish flu caused by the H1N1 strain of influenza A virus that occurred in 1918 was epidemic and spread all over the world. The total number of deaths from influenza in Spain was 147,114 in 1918 and 21,235 in 1919 (Trilla, A., Trilla, G., & Daer, 2008). The epidemics of influenza A virus have been occurred several times from 1918 until now, for example; the epidemic of influenza caused by a variety strains of influenza A virus H1N1 (Spanish influenza) in 1918, H2N2 (Asian influenza) in 1957, and H3N2 (Hong Kong Influenza) in 1968. In 2004, there was an outbreak of the H5N1 (Bird Flu) virus, and the first pandemic virus was spread to humans.

In 2009, there was an epidemic of H1N1 (Swine flu) which is spread from pigs to people (Morens & Taubenberger, 2011; Neumann, Noda, & Kawaoka, 2009).

2.4 Transmission of Influenza Virus

Influenza virus can be transmitted among humans in three ways: 1) by direct contact with infected individuals; 2) by contact with contaminated objects (called fomites, such as toys, doorknobs); and 3) by inhalation of virus-laden aerosols (Figure 2.3).

Influenza virus can be transmitted by inhalation of nasal mucous, saliva, coughing, sneezing, talking and phlegm of patients who cough or sneeze and hand contact as well as using various appliances together with patients such as towel, glasses, telephone and toys (Paules & Subbarao, 2017).

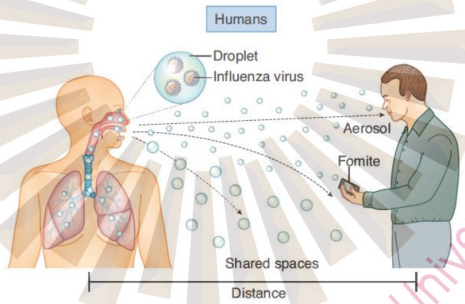


Figure 2.3 Influenza virus: Transmission channels

Source: Harow, 2015

2.5 Clinical Manifestation of Influenza Virus

The expression of influenza virus infection varies from asymptomatic to severe symptoms. The severity of the illness depends on both the host and the virus. The symptoms of influenza virus will appear after the incubation period of 1-2 days, with symptoms such as headache, malaise, myalgia, fever, chills, coughing, sneezing, nasal sore throat. and anorexia for about 3-8 days. Cough and malaise can last up to 2 weeks (Paules & Subbarao, 2017) (Figure 2.4).

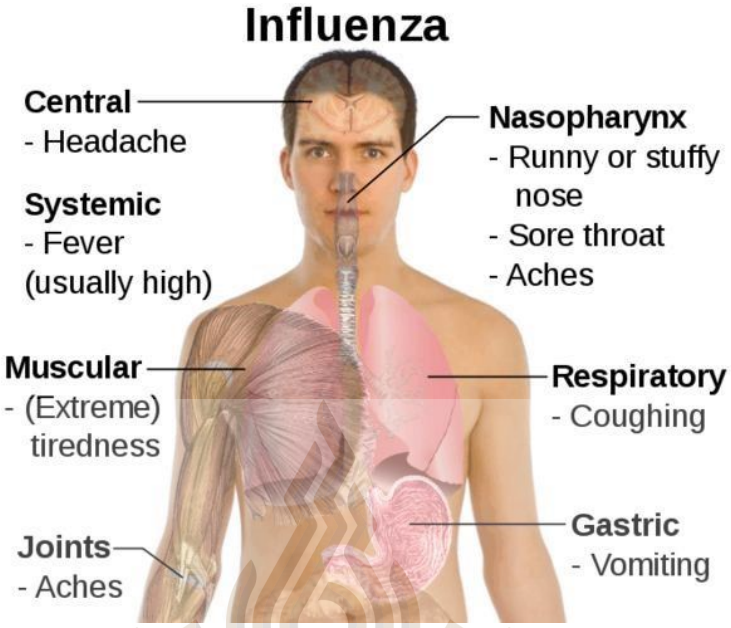


Figure 2.4 Symptoms of Influenza virus

Source: Akoaypilipino, 2019

2.6 Prevention and Control of Influenza Virus Infection

Influenza virus can be found at any age, mostly in children. But the mortality rate occurs with patients older than 65 years or those with underlying diseases such as heart disease, lung disease, and kidney disease (Paules & Subbarao, 2017). The most effective prevention methods for the prevention of Influenza virus infection are not sharing things with others, washing your hands frequently, not getting in touch with patients with Influenza virus symptoms, avoiding being in a crowded place and bad weather, and vaccination against Influenza virus. These methods can reduce the infection rate and reduce the spread of the Influenza virus as well as complications of Influenza virus (Figure 2.5).

Figure 2.5 Prevention and control of Influenza virus

Source: Aravatla Phc (Govt. Primary Health Centre), 2018

2.7 Diagnosis of Influenza Virus Infection

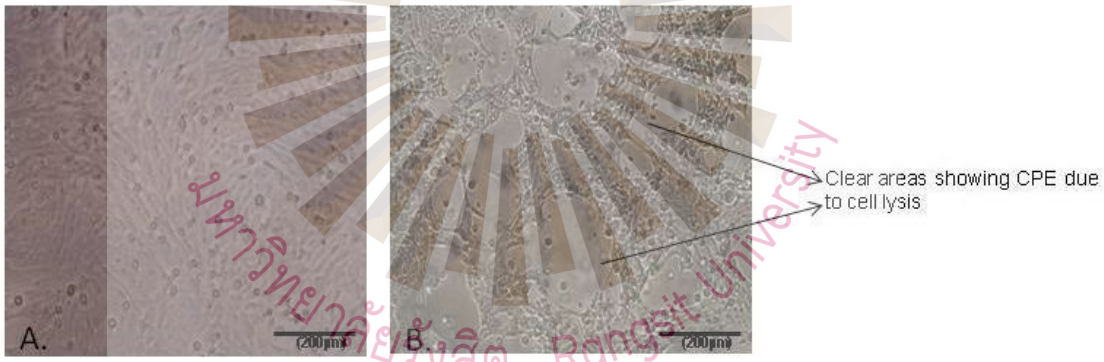
Influenza virus is a major cause of respiratory infections. Several reports in 2008 revealed that approximately 90 million people were infected with new Influenza viruses around the world and approximately people around 28,000-111,500 died from these infections (Nair et al., 2011). Therefore, to reduce and prevent Influenza outbreaks, promptly diagnosis of Influenza virus is important. In addition, investigations for infectious agents aid in the diagnosis and can be used to guide appropriate treatment and provide information for influenza surveillance. Laboratory tests for Influenza virus include viral culture, viral antigen detection, and nucleic acid testing. Each method has different advantages and disadvantages (Henrickson & Hall, 2007; Landry, 2011).

2.7.1 Cell Culture

The isolation of the virus in cell culture is a gold standard method and is effective because the isolated virus can be classified into types. In addition, the antigenicity and the genetic properties of the virus are also known. This method is also used for vaccine preparation.

The virus was cultured in Madin-Darby cells (MDCK) and then observed the changes in the shape of the MDCK cells. MDCK cells begin to look round, forming small groups and gradually expanding into clusters, called cytopathic effect (CPE) (Genzel, Olmer, Schäfer, and Reichl, 2006) (Figure 2.6).

The disadvantage of cell culture is that it takes 3-4 days to get the result (Paules & Subbarao, 2017).



A. Normal cell line B. Cell line showing cytopathic effect (CPE)

Figure 2.6 Influenza virus culture in MDCK cell line

Source: ResearchGate GmbH, 2017

2.7.2 Immunofluorescence Assay (IFA)

Immunofluorescence assay is a test that fluorescent substance is labeled with antigen or antibody for detection of antigen or antibody, respectively. For Influenza viral antigen detection,

the specimen was placed on the slide and fixed with acetone, then stained with specific influenza antibodies conjugated to fluorescence. The fluorescence conjugated to antibody coupled with viral antigen was examined under the fluorescence microscope (Noyola et al., 2000).

The advantage of IFA is the short turnaround time. However, the sensitivity and specificity of this method depend on antibodies used in the assay and this method requires an expert technician (Dwyer, Smith, Catton, & Barr, 2006; Paules & Subbarao, 2017).

2.7.3 Rapid Antigen Detection

The influenza viral antigen detection method widely used is an immuno-chromatographic test. This test is based on chromatography together with the reaction between antigen and antibody by applying different dissolved and absorbed substances. The antigen or antibody labeled with colored substances such as colloidal gold-coated at the starting position test will be used as a mobile phase. Nitrocellulose membrane, also known as the immobilized phase, is the area to detect the reaction between antigen and antibody in the fixation. In the strip, there will be a display bar that is coated with at least 2 tabs; 1) A control line is a position that shows the quality of assays in which the control line must always appear. 2) Test line is the result of the test (Vaughn et al., 1998; Wang, Hou, & Ma, 2015).

The advantages of rapid antigen detection are a short turnaround time and no specialized equipment required. However, this method reveals low sensitivity and low specificity (Dwyer et al., 2006; Paules & Subbarao, 2017).

2.7.4 Molecular Technique

At present, studies in molecular biology have progressed rapidly. Many techniques assist in the detection of DNA or RNA. They are used in the diagnosis and isolation of the virus. In each technique, there are some advantages such as reducing turnaround time (takes about 4-6 hours to test), high sensitivity and specificity, while the disadvantage is expensive. There are many

molecular techniques such as polymerase chain reaction (PCR) and Reverse transcription PCR. Currently, PCR is a commonly used and indispensable technique in medical and research laboratories. PCR is the process of synthesizing DNA in vitro in a short time and multiplying DNA (Paules & Subbarao, 2017).

2.8 Nucleic Acid Extraction

Genetic material from viral particles is extracted and used in the study of biomolecular techniques. The principle of genetic material extraction is to obtain pure genetic material molecules.

2.8.1 Automated Nucleic Acid

Currently, genetic extraction tools are developed as automated tools for DNA or RNA extraction to make this genetic material of better quality and purity. Using an automatic extractor, the quality of pure nucleic acid can be high. In addition, using an automatic extractor can command the machine to work from start to finish, reduce labor, turn-around time, error control, and safeguard against contamination. There are many brands of automatic extractors and many companies, such as QIA symphony from the QIAGEN, AnaPrep 12 automated nucleic acid preparation instrument from the BioChain, InnuPure® C16 automated extraction system from the *Analytik Jena or* eMAG™ nucleic acid extraction system from the bioMérieux.

2.8.2 EASYMAG@ Nucleic Acid Extraction System

EASYMAG@ instrument is designed to meet the most critical needs when it comes to premium quality total nucleic acid extraction. The system automates an enhanced magnetic silica version of BOOM technology, a gold standard for efficient universal extraction of RNA and DNA. The system offers ease-of-use and optimal workflow for enhancing productivity consists of four major steps (Figure 2.7)

2.8.2.1 Lysis step: It breaks the cell particles by using a lysis buffer. The lysis buffer breaks down the cell wall to rupture the cell virus particles.

2.8.2.2 Binding step: During incubation of the lysed sample, all the target nucleic acid is captured by magnetic silica particles.

2.8.2.3 Washing step: The buffer solution is added to wash away the nucleic acid enabling the system to purify the nucleic acids through several washing steps.

2.8.2.4 Elution step: In this last step, the magnetic silica particles are separated from the nucleic acid by elution buffer.

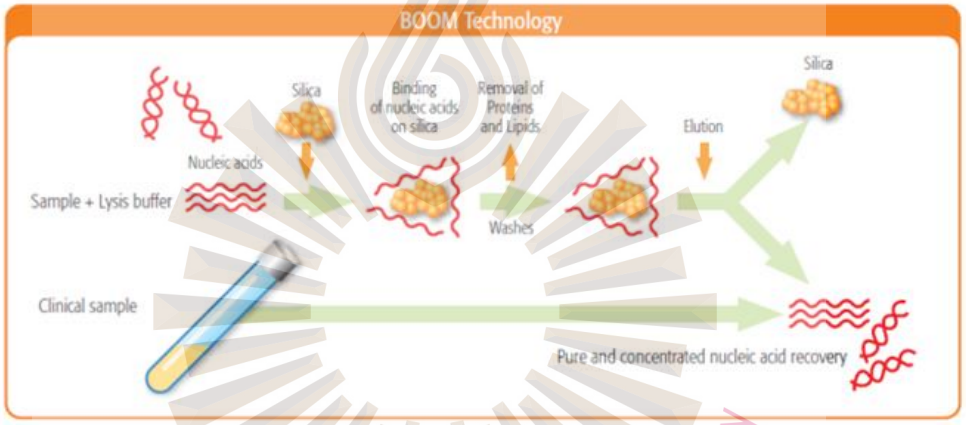


Figure 2.7 Principle of the BOOM technology in the EASYMAG.

Source: Biomérieux Asean Website, 2018

2.8.3 Solana Influenza A+B assay

Solana Influenza A+B assay test is used to detect and differentiate Influenza A and Influenza B viruses from patients with respiratory tract infections by using the Isothermal reverse transcriptase-helicase dependent amplification principle (RT-HAD). In the Solana Influenza A + B assay, the reaction tube is packed with RT-HAD reagents, dNTPs, primers, and probes in lyophilized form. When the samples are put in the reaction tube and are incubated at 95°C, then they are placed in Solana machine to increase the number and the specific location of the Influenza A and Influenza B virus are detected by primers and reported by fluorescence probes specific to Influenza A and Influenza B. In the process, a buffer tube containing a competitive

process control (PRC) is used for testing for the workflow, distinguishing inhibitors in the specimens, and checking for the deterioration of reagents and equipment. The PRC is added to the primers specific to the Influenza B virus, then Influenza B virus is detected by fluorescence probes specific to PRC. The target probes of both strains of the Influenza A and B viruses and the PRC probe at one end are labeled with a quencher on one side and other side is labeled with fluorophore. In the annealing phase, the fluorophore is cut by RNaseH2 and the fluorophore signal is increased due to the fluorophore and quencher being separated from each other.

Solana Influenza A + B assay is easy to use, fast in the detection of Influenza A and B viruses and inexpensive. Therefore, it may be suitable for using in the diagnosis of Influenza virus as an aid clinician making the decision and treatment of the patients.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Solana Influenza A+B assay (Quidel Corporation, OH, USA)

3.1.2 Lyra Influenza A+B Real-Time PCR (Quidel Corporation, OH, USA)

3.1.3 Nasal Swab and Nasopharyngeal Swab

3.2 Methods

3.2.1 Samples and Sample preparation

A total of two hundred and sixty nasal swabs and nasopharyngeal swabs used in this study were kindly provided by the Virology Laboratory, Department of Pathology, Faculty of Medicine Ramathibodi Hospital. Two hundred and forty nasal swabs and nasopharyngeal swabs were obtained from suspected Influenza virus infection patients while twenty samples were obtained from other respiratory tract infections besides Influenza virus. The samples were stored at -20°C. The samples were thawed at room temperature before testing (Protocol number 1303).

3.2.2 Solana Influenza A+B assay

Fifty microliters of sample was added to 1.5 ml viral transport media (VTM) in the process buffer tube. The tube was then mixed in the vortex mixer for 5 seconds and incubated at 95°C for 5 minutes to break the cells.

Fifty microliters of the process buffer were added to the reaction tube containing a competitive process control (PRC) and the reaction tube was put into the Solana machine. At this step viral genetic material was amplified (Figure 3.1 and Figure 3.2).

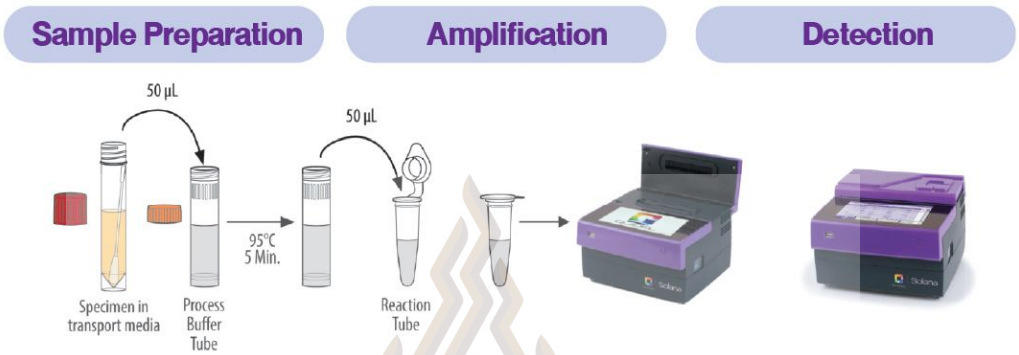


Figure 3.1 Solana Influenza A+B workflow

Source: Quidel Corporation provides CLSI, 2020

Figure 3.2 The status of all samples during the run will be “pending”

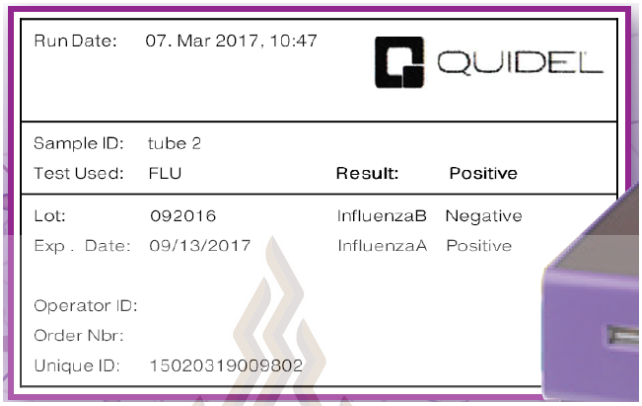
Source: Quidel Corporation provides CLSI, 2020

3.2.3 Interpretation of results Influenza A+B assay

Solana processed for testing of Influenza A and Influenza B automatically. The positive results of the test indicated that the RNA detection of Influenza virus was possible and the negative results

indicated that there was no viral RNA detected, while the PRC was detected. The device displayed invalid only when Influenza A, Influenza B, and PRC were not detected (Figure 3.3).

A)



B)

Figure 3.3 The report of the result of Solana Influenza A+B assay

A) Printing report of the result. B) Showing results from the Solana screen.

Source: Quidel Corporation provides CLSI, 2020

3.2.4 Real-Time Reverse Transcriptase PCR

3.2.4.1 RNA Extraction

Influenza viral RNA was extracted from nasal swabs and nasopharyngeal swabs using BOOM technology in the eMAG instrument (Biomerieux Italia SpA, Italy). Two hundred microliters of sample were mixed with 1000 µl lysis buffer into the vessels. The mixture was well mixed up and down by pipette, then incubated at room temperature for 10 minutes. Then the

extracted RNA was dissolved in the vessel and carefully transferred to new tubes and stored at -20 ˚C until used for real-time RT-PCR.

3.2.4.2 Master Mix Preparation

Mastermix preparation for the Lyra real-Time PCR Influenza A+B detection kit contained 15 µl of rehydration of lyophilized master mix (Buffer, Mg^{2+}, dNTP, and *Taq* polymerase) and 5 µl of nucleic acid template or internal positive control or internal negative control. The details of master mix preparation showed in Table 3.1.

Table 3.1 Master mix preparation (Lyra Real-time PCR System)

Reagents	1x Reaction
Master mix	15 µl
Extracted sample	5 µl
Total	20 µl

3.2.4.3 RNA amplification

The Real-Time PCR conditions were the initial reverse transcription at 55 ºC for 5 minutes, at 60 ºC for 5 minutes and at 65 ºC for 5 minutes followed by amplification at a temperature of 92 ºC for 5 seconds, at 57 ºC for 40 seconds, at 92 ºC for 5 seconds and at 57 ºC for 40 seconds. Then it was entranced to PCR cycling for 35 cycles at 92 ºC for 5 seconds and at 57 ºC for 40 seconds. (Figure 3.4) The RNA was amplified using Lyra Real-Time PCR (Quidel Corporation, OH, USA).

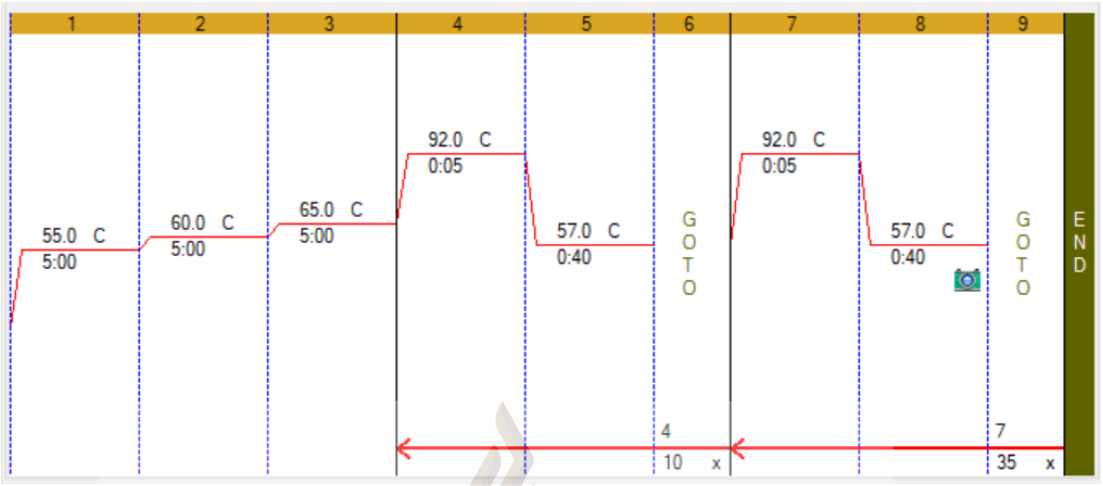


Figure 3.4 Real-Time PCR conditions

3.2.4.4 Interpretation of results

Real-time PCR results were represented on the amplification curve after the RNA amplification. The amplification curve is determined with the Ct (Threshold Cycle) value. It was an intersection point between the fluorescence represented on the Y-axis and the baseline and automatically calculated the software system in the real-time PCR machine. The baseline was parallel with the number of PCR cycles in the X-axis. The interpretation of the results was based on the amplification curve to compare to the positive control and negative control (or NTC) (Table 3.2).

Table 3.2 Interpretation of results

Assay Result	Detector: Influenza A	Detector: Influenza B
Negative	Ct<5.0	Ct<5.0
Influenza A positive	5.0≤Ct≤35.0	Ct<5.0
Influenza B positive	Ct<5.0	5.0≤Ct≤35.0
Invalid	Ct<5.0 or Ct>35.0	Ct<5.0 or Ct>35.0

3.2.5 Outcome measurement

The effectiveness of Solana Influenza A+B assay to detect and differentiate Influenza A and Influenza B viral RNA was evaluated by determination some parameters such as accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and limited of detection (LOD).

3.2.5.1 Sensitivity is the proportion of patients with the disease and all test results are positive.

$$\text{Sensitivity} = \frac{\text{True positive (TP)}}{\text{True positive + False negative (FN)}} \text{X100}$$

3.2.5.2 Specificity is the proportion of patients who do not have the disease and all test results are negative.

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative + False positive}} \text{X100}$$

3.2.5.3 Positive predictive value (PPV) means the percentage of positive results in the test, which is a true positive result.

$$\text{Positive predictive value} = \frac{\text{True positive}}{\text{True positive + False positive}} \text{X100}$$

3.2.5.4 Negative predictive value (NPV) means the percentage of negative results in the test, which is the actual negative result.

$$\text{Negative predictive value} = \frac{\text{True negative}}{\text{True negative} + \text{False negative}} \text{ X100}$$

3.2.5.5 Accuracy is the accuracy of the measurement method of analysis, the value closest to the true value.

$$\text{Accuracy} = \frac{\text{True positive} + \text{True negative}}{\text{True positive} + \text{True negative} + \text{False positive} + \text{False negative}} \text{ X100}$$

3.2.5.6 Limit of detection (LOD) is the lowest amount of infection this test can detect.

3.3 Ethics Statement

This study was approved by the Ethical Clearance Committee on Human Right Related to Research Involving Human Subjects Faculty of Medicine Ramathibodi Hospital, Mahidol University. The protocol number is ID 1303.

CHAPTER 4

RESULTS

4.1 Sample Size

The sample size was obtained to assess sensitivity and specificity from the following formula (Hajian-Tilaki, 2014).

$$n_{Se} = \frac{Z^2_{\alpha/2}\, Se(1-Se)}{d^2 \times Prev}$$

Se = Sensitivity of previous research

prev = Pre-determined value of sensitivity

d = Estimation error

α = 0.05 ($Z\alpha/2 = 1.96$)

Based on the preliminary study, the test sensitivity of the previous study was 83.3% (Old & Kidd,2020), calculated for 95% confidence interval (α = 0.05), d = 0.1 and prev = 0.2464 (Eamchotchawalit, Piyaraj, Narongdej, Charoensakulchai, and Chanthowong, 2014). Therefore, the sample size of this study was 220.

$$n_{Se} = \frac{(1.96)^2(0.833)(0.17)}{0.1^2 \times 0.2464} = 220$$

From the calculation of the sample size, it should not be less than 220 samples, so a total of 260 samples were used.

4.2 The diagnostic performance evaluation

A total of 260 samples were tested for Influenza A and B virus with Solana Influenza A+B and Real-Time PCR as a reference method. The results from both techniques showed that 91 samples and 99 samples were positive for Influenza A and Influenza B, respectively, while 70 samples were negative for both Influenza A and Influenza B as shown in Table 4.1

Table 4.1 The results of samples tested with real-time RT-PCR and Solana influenza A+B

Clinical Results	Number (samples)
Positive with both real-time RT-PCR and Solana influenza A	91
Positive with both real-time RT-PCR and Solana influenza B	99
Negative with both real-time RT-PCR and Solana influenza A+B	70
Positive with Solana influenza A+B only	0
Positive with RT-PCR only	0

The results from Solana influenza A+B were calculated for the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, and limit of detection (LOD).

The sensitivity is calculated by substituting true positive and false negative in the formula of sensitivity shown below.

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100$$

The study showed that all 91 samples from Influenza A infected patients and 99 samples from Influenza B infected patients had positive results with both the Solana Influenza A+B isothermal amplification method and RT-PCR. In addition, there was no false-negative result.

Therefore, the sensitivity of Solana Influenza A+B isothermal amplification for the detection of Influenza A and Influenza B compared to RT-PCR was 100%.

$$\text{Sensitivity (Influenza A)} = \frac{91}{91+0} \times 100 = 100$$

$$\text{Sensitivity (Influenza B)} = \frac{99}{99+0} \times 100 = 100$$

The specificity is calculated by substituting true negative and false positive in the formula of specificity shown below.

$$\text{Specificity} = \frac{TN}{TN+FP} \times 100$$

The study showed that all 70 negative samples investigated by Solana Influenza A+B isothermal amplification method were not positive by RT-PCR. In addition, 20 samples from other respiratory infection patients besides Influenza A and Influenza B also showed negative results with Solana Influenza A+B isothermal amplification. Therefore, the specificity of Solana Influenza A+B isothermal amplification for the detection of Influenza A and Influenza B compared to RT-PCR was 100%.

$$\text{Specificity (Influenza A)} = \frac{70}{70+0} \times 100 = 100$$

$$\text{Specificity (Influenza B)} = \frac{70}{70+0} \times 100 = 100$$

Positive predictive value (PPV) is calculated by substituting true positive and false positive in the formula of PPV shown below.

$$PPV = \frac{TP}{TP+FP} \times 100$$

The study showed that all 91 samples from Influenza A infected patients and 99 samples from Influenza B infected patients had positive results with both the Solana Influenza A+B isothermal amplification method and RT-PCR. In addition, there was no false-positive result. Therefore, the Positive predictive value (PPV) of Solana Influenza A+B isothermal amplification for the detection of Influenza A and Influenza B compared to RT-PCR was 100%.

$$PPV_{(\text{Influenza A})} = \frac{91}{91+0} \times 100 = 100$$

$$PPV_{(\text{Influenza B})} = \frac{99}{99+0} \times 100 = 100$$

Negative predictive value (NPV) is calculated by substituting true negative and false negative in the formula of NPV shown below.

$$NPV = \frac{TN}{TN+FN} \times 100$$

The study showed that all 70 negative samples investigated by Solana Influenza A+B isothermal amplification method were not positive by RT-PCR. In addition, 20 samples from other respiratory infection patients besides Influenza A and Influenza B also showed negative results with Solana Influenza A+B isothermal amplification. Therefore, the negative predictive

value (NPV) of Solana Influenza A+B isothermal amplification for the detection of Influenza A and Influenza B compared to RT-PCR was 100%.

$$\text{NPV (Influenza A)} = \frac{70}{70+0} \times 100 = 100$$

$$\text{NPV (Influenza B)} = \frac{70}{70+0} \times 100 = 100$$

Accuracy is calculated by substituting true positive, true negative, false positive, and false negative in the formula of accuracy shown below.

$$\text{Accuracy} = \frac{TP+TN}{TP+FP+FN+TN} \times 100$$

The study showed that all 91 samples from Influenza A infected patients and 99 samples from Influenza B infected patients had positive results with both the Solana Influenza A+B isothermal amplification method and RT-PCR. In addition, all 70 negative samples investigated by Solana Influenza A+B isothermal amplification method were not positive by RT-PCR. There was no false positive or false negative result. Therefore, the accuracy of Solana Influenza A+B isothermal amplification for the detection of Influenza A and Influenza B compared to RT-PCR was 100%.

$$\text{Accuracy} = \frac{190+50}{190+0+0+50} \times 100$$

4.3 The results of the Cross-Reactivity

Twenty samples containing any viruses other than Influenza A and Influenza B virus were tested using Solana Influenza A+B isothermal amplification method for cross-reactivity test. The results showed that Influenza A and Influenza B virus were not detected in those samples by Solana Influenza A+B isothermal amplification method as shown in Table 4.2

Table 4.2 Virus used to determine reaction profile of the assay

Clinical sample number	Pathogen list	Number of specimens	Results
1	Human rhinoviruses	4	Negative
2	Human respiratory syncytial viruses A	3	Negative
3	Human respiratory syncytial viruses B	3	Negative
4	Human coronaviruses 229E	4	Negative
5	Human coronaviruses OC43	4	Negative
6	Human metapneumoviruses	2	Negative

4.4 The limited of detection

The positive control sample containing Influenza virus type A or B (Amplirun total Flu A/Flu B/RSV) was serially diluted to determine the limit of detection. The result showed that Solana Influenza A+B isothermal amplification method PCR can detect Influenza A virus and Influenza A virus at least 30 µl (1,080 copies/µl) and 2.5 µl (115 copies/µl), respectively as shown in Table 4.3

Table 4.3 Quantitative data of limited of detection

Positive control (µl)	Transport media (µl)	Influenza A (copies/ul)	Influenza A (copies/ml)	Influenza B (copies/ul)	Influenza B (copies/ml)	Result (Influenza A)	Result (Influenza B)
50	0	1800	$1.8*10^3$	2300	$2.3*10^3$	Positive	Positive
40	10	1440	$1.44*10^3$	1840	$1.84*10^3$	Positive	Positive
30	20	1080	$1.08*10^3$	1380	$1.38*10^3$	Positive	Positive
20	30	720	$7.2*10^2$	920	$9.2*10^2$	Negative	Positive
10	40	360	$3.6*10^2$	460	$4.6*10^2$	Negative	Positive
5	45	180	$1.8*10^2$	230	$2.3*10^2$	Negative	Positive
2.5	47.5	90	$9*10^1$	115	$1.15*10^2$	Negative	Positive
1.25	48.75	45	$4.5*10^1$	57.50	$5.75*10^1$	Negative	Negative
0.625	49.375	23	$2.3*10^1$	28.75	$2.875*10^1$	Negative	Negative
Copies/vials						18,000	23,000
Copies/ml						36,000	46,000
Copies/µl						36	46
Add 500 µl of Molecular Biology grade water to vial							

CHAPTER 5

DISCUSSION AND CONCLUSION

Influenza is one of the most important respiratory tract infections due to the emergence of a pandemic many times. Clinical manifestation of this disease ranges from asymptomatic to fulminant. In addition, the complications of this disease lead to other viral and bacterial pneumonia, acute respiratory distress syndrome, encephalopathy, myocarditis, pericarditis, and myositis which can cause high mortality rate especially in very young and elderly people (Lowen et al., 2007; Nayak et al., 2004). Clinical diagnosis of influenza is difficult because symptoms are varied and have common characteristics with other respiratory viruses

In the diagnosis of infectious diseases, clinical manifestations often take precedence, but sometimes similar clinical manifestations cannot provide an accurate diagnosis. Therefore, additional laboratory examinations are required. The capacity of the laboratory will vary from area to area. If a simple diagnostic tool can be done at the point of care and get results in a short time, it will be very useful in the treatment.

The Solana Influenza A+B assay is considered as a point-of-care testing (PoCT). It is a test to provide point-of-care diagnostics that currently includes a rapid-result test and can be done outside the sophisticated central laboratory (Moore, 2013). This assay has the objective to provide diagnosis, follow-up, and treatment, and infectious control. This test can be performed at home, outpatient examination room, primary hospital as well as a secondary hospital which does not require complicated tools. Therefore, it may be very useful in developing countries. The rapid diagnosis of influenza may help in the management of patients and lower overall treatment costs. The Solana Influenza A+B assay is a rapid diagnostic assays and is relatively simple to perform which can produce results within forty minutes. In addition, this study suggested that the Solana

influenza A+B assay has high sensitivity and specificity compared to Lyra Influenza A+B Assay (Real-Time PCR).

This study revealed that the Solana Influenza A+B assay displayed high sensitivity detection for both Influenza A and Influenza B compared to the Real-Time PCR. No false negative and positive results were found. Therefore, test results obtained from the Solana Influenza A+B assay were substantial as well as Real-Time PCR. The Solana Influenza A+B assay takes a time only forty minutes while Real-Time PCR runs the test for more than four hours.

Influenza is a serious public health concern associated with high mortality. The rapid diagnostic test showed fast and accurate diagnostic of Influenza is essential for the administration of appropriate treatment. The sensitivity and specificity of a rapid diagnostic tests can report the results faster than a Real-Time PCR principle assay. The Solana Influenza A+B assay showed comparative results with Lyra Influenza A+B assay (Real-Time PCR kit). The Solana Influenza A+B assay is a rapid qualitative in vitro diagnostic test for the detection and differentiation of Influenza A and Influenza B viral RNA. The Solana Influenza A+B assay amplifies and detected the target sequences specific to Influenza A and/or Influenza B using Isothermal Reverse Transcriptase-Helicase-Dependent Amplification (RT_HDA) in the presence of target-specific fluorescence probes which is performed in the Solana instrument. The finding suggested that a fluorescence-based immunoassay is more sensitive than the gold conjugate.

In addition, Influenza A and Influenza B virus were not detected in 20 samples containing any viruses other than Influenza A and Influenza B virus when tested with Solana Influenza A+B isothermal amplification method. Therefore, this method showed no cross-reaction with viruses other than Influenza A and Influenza B virus.

In conclusion, the commercially available in vitro diagnostic Solana Influenza A+B assay shows high a potential tool for the detection of seasonal influenza compared to Lyra Influenza A+B assay (Real-Time PCR kit). It is one of the attractive platforms for a clear diagnosis of Influenza. The assay detects viral nucleic acids that have been extracted from patient samples. A

multiplex reaction is carried out under optimized conditions in a single tube for each of the detection of the target viruses present in the sample. This rapid multiplex real-time polymerase chain reaction (PCR) has high sensitivity and high specificity diagnostic techniques. In addition, the advantages of using Solana Influenza A+B are its low cost, reduced sample extraction process, and low turn-around time. It only takes 40 minutes compared to real-time PCR, which takes at least 4 hours to complete.

REFERENCES

Akoaypilipino. (2019). *Mga Dapat Malaman Tungkol Sa Trangkaso At Sa Bakuna Nito*. Retrieved from https://www.akoaypilipino.eu/gabay/mga-dapat-malaman-tungkol-sa-bakuna-sa-trangkaso/

Aravatla Phc (Govt. Primary Health Centre). (2018, October 29). Swine Flu Poster Tamil [Web log message]. Retrieved from http://aravatlaphc.blogspot.com/2018/10/

Biomérieux Asean Website. (2018). *First in Boom Technology*. Retrived From https://www.biomerieux-asean.com/product/nuclisensr-easymagr

Bouvier, N. M., & Palese, P. (2008). The biology of influenza viruses. *Vaccine*, *26*, D49–D53. https://doi.org/10.1016/j.vaccine.2008.07.039

Centers for Disease Control and Prevention. (2021). *Types of Influenza Viruses*. Retrieved from https://www.cdc.gov/flu/about/viruses/types.htm

Collin, E. A., Sheng, Z., Lang, Y., Ma, W., Hause, B. M., & Li, F. (2015). Cocirculation of Two Distinct Genetic and Antigenic Lineages of Proposed Influenza D Virus in Cattle. *Journal of Virology*, *89*(2), 1036–1042. https://doi.org/10.1128/JVI.02718-14

Dwyer, D. E., Smith, D. W., Catton, M. G., & Barr, I. G. (2006). *Laboratory diagnosis of human seasonal and pandemic influenza virus infection*, *185*(10), 848-853.

Eamchotchawalit, T., Piyaraj, P., Narongdej, P., Charoensakulchai, S., & Chanthowong, C. (2014). Epidemiology of influenza: findings from surveillance system of 40 hospital in Thailand, 2010-2019. *European Journal of Public Health, 30*(Supplement_5), ckaa165.1098. https://doi.org/10.1093/eurpub/ckaa165.1098

Fouchier, R. A. M., Munster, V., Wallensten, A., Bestebroer, T. M., Herfst, S., Smith, D., . . . Osterhaus, A. D. M. E. (2005). Characterization of a Novel Influenza A Virus Hemagglutinin Subtype (H16) Obtained from Black-Headed Gulls. *Journal of Virology*, *79*(5), 2814–2822. https://doi.org/10.1128/JVI.79.5.2814-2822.2005

Francis, T. (1935). IMMUNOLOGICAL STUDIES WITH THE VIRUS OF INFLUENZA. *Journal of Experimental Medicine*, *62*(4), 505–516. https://doi.org/10.1084/jem.62.4.505

Francis, T. (1937). Epidemiological Studies in Influenza. *American Journal of Public Health and the Nations Health*, *27*(3), 211–225. https://doi.org/10.2105/AJPH.27.3.211

REFERENCES (CONT.)

Genzel, Y., Olmer, R. M., Schäfer, B., & Reichl, U. (2006). Wave microcarrier cultivation of MDCK cells for influenza virus production in serum containing and serum-free media. *Vaccine*, *24*(35–36), 6074–6087. https://doi.org/10.1016/j.vaccine.2006.05.023

Green, N., Alexander, H., Olson, A., Alexander, S., Shinnick, T. M., Sutcliffe, J. G., & Lerner, R. A. (1982). Immunogenic structure of the influenza virus hemagglutinin. *Cell*, *28*(3), 477–487. https://doi.org/10.1016/0092-8674(82)90202-1

Hajian-Tilaki, K. (2014). Sample size estimation in diagnostic test studies of biomedical informatics. *Journal of biomedical informatics, 48*(1), 193-204.

Harow, C. (2015). *Influenza Virus: Transmission Channels*. Retrieved from https://coryharow.wordpress.com/2015/11/18/influenza-virus-transmission-channels/

Henrickson, K. J., & Hall, C. B. (2007). Diagnostic Assays for Respiratory Syncytial Virus Disease: *The Pediatric Infectious Disease Journal*, *26*(Supplement), S36–S40. https://doi.org/10.1097/INF.0b013e318157da6f

Landry, M. L. (2011). Diagnostic tests for influenza infection: *Current Opinion in Pediatrics*, *23*(1), 91–97. https://doi.org/10.1097/MOP.0b013e328341ebd9

Lowen, A. C., Mubarekai, S., Steel, J., & Palese, P. (2007). Influenza virus transmission is dependent on relative humidity and temperature. *PLoS Pathog, 3*(10), 1470–1476. doi:10.1371/journal.ppat.0030151

Matsuzaki, Y., Katsushima, N., Nagai, Y., Shoji, M., Itagaki, T., Sakamoto, M., . . . Nishimura, H. (2006). Clinical Features of Influenza C Virus Infection in Children. *The Journal of Infectious Diseases*, *193*(9), 1229–1235. https://doi.org/10.1086/502973

Medina, R. A., & García-Sastre, A. (2011). Influenza A viruses: New research developments. *Nature Reviews Microbiology*, *9*(8), 590–603. https://doi.org/10.1038/nrmicro2613

Moore, C. (2013). Point-of-care tests for infection control: Should rapid testing be in the laboratory or at the front line? *Journal of Hospital Infection*, *85*(1), 1–7. https://doi.org/10.1016/j.jhin.2013.06.005

REFERENCES (CONT.)

Morens, D. M., & Taubenberger, J. K. (2011). Pandemic influenza: Certain uncertainties: Pandemic influenza. *Reviews in Medical Virology, 21*(5) 262-284. https://doi.org/10.1002/rmv.689

Nair, H., Brooks, W. A., Katz, M., Roca, A., Berkley, J. A., Madhi, S. A., . . . Campbell, H. (2011). Global burden of respiratory infections due to seasonal influenza in young children: A systematic review and meta-analysis. *The Lancet*, *378*(9807), 1917–1930. https://doi.org/10.1016/S0140-6736(11)61051-9

Nayak, D. P., Hui, E. K, & Barman, S. (2004). Assembly and budding of influenza virus. *Virus Research, 106*(2004), 147–165.

Neumann, G., Noda, T., & Kawaoka, Y. (2009). Emergence and pandemic potential of swine-origin H1N1 influenza virus. *Nature*, *459*(7249), 931–939. https://doi.org/10.1038/nature08157

Noyola, D. E., Clark, B., O'Donnell, F. T., Atmar, R. L., Greer, J., & Demmler, G. J. (2000). Comparison of a New Neuraminidase Detection Assay with an Enzyme Immunoassay, Immunofluorescence, and Culture for Rapid Detection of Influenza A and B Viruses in Nasal Wash Specimens. *J. CLIN. MICROBIOL.*, *38*(3), 1161-1165.

Old, T., & Kidd, S. (2020). Clinical evaluation of the quidel solana flu a + b assay in a uk district general hospital laboratory. *Journal of Infection and Public Health*, *13*(2), 357. https://doi.org/10.1016/j.jiph.2020.01.137

Paules, C., & Subbarao, K. (2017). Influenza. *The Lancet*, *390*(10095), 697–708. https://doi.org/10.1016/S0140-6736(17)30129-0

Qi, L., Kash, J. C., Dugan, V. G., Jagger, B. W., Lau, Y.-F., Sheng, Z.-M., . . . Taubenberger, J. K. (2011). The ability of pandemic influenza virus hemagglutinins to induce lower respiratory pathology is associated with decreased surfactant protein D binding. *Virology*, *412*(2), 426–434. https://doi.org/10.1016/j.virol.2011.01.029

Quidel Corporation provides CLSI. (2020). *FSolana Influenza A+B Assay CLIA Complexity: Moderate*. Retrived from https://www.quidel.com/sites/default/files/product/documents/CLM300000EN00.pdf

REFERENCES (CONT.)

ResearchGate GmbH. (2017). *Comparative Analysis of Molecular Methods for Detection of Influenza Viruses*. Retrieved from https://www.researchgate.net/figure/Influenza-virus-culture-in-MDCK-cell-line-A-Normal-cell-line-B-Cell-line-showing_fig1_321906384

Rosenthal, P. B., Zhang, X., Formanowski, F., Fitz, W., Wong, C.-H., Meier-Ewert, H., . . . Wiley, D. C. (1998). Structure of the haemagglutinin-esterase-fusion glycoprotein of influenza C virus. *Nature*, *396*(6706), 92–96. https://doi.org/10.1038/23974

Scholtissek, C., Rohde, W., Von Hoyningen, V., & Rott, R. (1978). On the origin of the human influenza virus subtypes H2N2 and H3N2. *Virology*, *87*(1), 13–20. https://doi.org/10.1016/0042-6822(78)90153-8

Sreenivasan, C., Thomas, M., Sheng, Z., Hause, B. M., Collin, E. A., Knudsen, D. E. B., . . . Li, F. (2015). Replication and Transmission of the Novel Bovine Influenza D Virus in a Guinea Pig Model. *Journal of Virology*, *89*(23), 11990–12001. https://doi.org/10.1128/JVI.01630-15

Suarez, D. L., & Schultz-Cherry, S. (2000). Immunology of avian in¯uenza virus: A review. *Developmental and Comparative Immunology, 24*(2000), 269-283.

Taubenberger, J. K., & Morens, D. M. (2010). Influenza: The Once and Future Pandemic. *Public Health Reports*, *125*(3_suppl), 15–26. https://doi.org/10.1177/00333549101250S305

Trilla, A., Trilla, G., & Daer, C. (2008). The 1918 "Spanish Flu" in Spain. *Clinical Infectious Diseases*, *47*(5), 668–673. https://doi.org/10.1086/590567

Uyeki, T. M. (2009). Human Infection with Highly Pathogenic Avian Influenza A (H5N1) Virus: Review of Clinical Issues. *Clinical Infectious Diseases*, *49*(2), 279–290. https://doi.org/10.1086/600035

Vaughn, D. W., Nisalak, A., Kalayanarooj, S., Solomon, T., Dung, N. M., Cuzzubbo, A., & Devine, P. L. (1998). Evaluation of a Rapid Immunochromatographic Test for Diagnosis of Dengue Virus Infection. *J. CLIN. MICROBIOL.*, *36*(1), 234-238.

REFERENCES (CONT.)

Wang, C., Hou, F., & Ma, Y. (2015). Simultaneous quantitative detection of multiple tumor markers with a rapid and sensitive multicolor quantum dots based immunochromatographic test strip. *Biosensors and Bioelectronics*, *68*(2015), 156–162. https://doi.org/10.1016/j.bios.2014.12.051

World Health Organication. (2021). *Percentage of people infected with Influenza viruses*. Retrieved from https://www.who.int/influenza/surveillance_monitoring/updates/latest_update_GIP_surveillance/en/

Wu, N. C., & Wilson, I. A. (2017). A Perspective on the Structural and Functional Constraints for Immune Evasion: Insights from Influenza Virus. *Journal of Molecular Biology*, *429*(17), 2694–2709. https://doi.org/10.1016/j.jmb.2017.06.015

Xu, X., Lindstrom, S. E., Shaw, M. W., Smith, C. B., Hall, H. E., Mungall, B. A., . . . Klimov, A. (2004). Reassortment and evolution of current human influenza A and B viruses. *Virus Research*, *103*(1–2), 55–60. https://doi.org/10.1016/j.virusres.2004.02.013

APPENDICES

APPENDIX A

RESULTS OF INFLUENZA A

Table 1 Results of Influenza A with Solana Influenza A+B assay compared with RT-PCR (No. 1-36)

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
1	V66-0554	A	11.75
2	V66-9716	A	33.96
3	V66-0616	A	21.81
4	V66-0326	A	12.04
5	V66-0529	A	25.46
6	V66-0819	A	22.91
7	V66-0777	A	32.13
8	V66-0569	A	17.03
9	V66-0434	A	20.99
10	V66-0132	A	17.75
11	V66-0573	A	13.22
12	V66-0586	A	19.17
13	V66-0547	A	18.15
14	V66-0447	A	22.35
15	V66-0463	A	12.38
16	V66-0667	A	22.18
17	V66-0613	A	17.49
18	V66-0596	A	16.69
19	V66-0566	A	21.93
20	V66-9963	A	10.18
21	V66-9962	A	20.03
22	V66-0435	A	28.09
23	V66-0329	A	14.16
24	V66-0561	A	16.05
25	V66-0321	A	25.33
26	V66-0384	A	16.89

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
27	V66-0623	A	13.47
28	V66-0811	A	27.31
29	V66-0636	A	8.22
30	V66-2959	A	15.91
31	V66-0803	A	8.02
32	V66-0752	A	19.16
33	V66-0702	A	18.49
34	V66-0688	A	14.59
35	V66-0672	A	12.65
36	V66-0682	A	16.07

Comments ; Ct = Threshold cycles

Table 2 Results of Influenza A with Solana Influenza A+B assay compared with RT-PCR (No. 37-72).

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
37	V66-0764	A	21.31
38	V66-0703	A	18.71
39	V66-0657	A	17.95
40	V66-0709	A	23.81
41	V66-9966	A	15.56
42	V66-2897	A	15.10
43	V66-8080	A	18.11
44	V66-0747	A	15.12
45	V66-2995	A	21.15
46	V66-2963	A	17.38
47	V66-2984	A	18.68
48	V66-2964	A	14.61

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
49	V66-2943	A	19.46
50	V66-2922	A	15.13
51	V66-2955	A	18.77
52	V66-2949	A	20.54
53	V66-2935	A	19.05
54	V66-2923	A	17.72
55	V66-2772	A	24.93
56	V66-2688	A	19.12
57	V66-2991	A	20.75
58	V66-2982	A	17.49
59	V66-2874	A	19.08
60	V66-2889	A	14.56
61	V66-2349	A	19.31
62	V66-2677	A	16.71
63	V66-2714	A	16.15
64	V66-2888	A	21.92
65	V66-2872	A	11.97
66	V66-2877	A	32.02
67	V66-2865	A	18.51
68	V66-2587	A	15.28
69	V66-2590	A	35.46
70	V66-2873	A	15.06
71	V66-2665	A	13.42
72	V66-2786	A	26.54

Comments ; Ct = Threshold cycles

Table 3 Results of Influenza A with Solana Influenza A+B assay compared with RT-PCR (No. 73-91).

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
73	V66-2809	A	18.96
74	V66-2817	A	24.20
75	V66-2827	A	15.57
76	V66-2656	A	20.68
77	V66-2722	A	18.12
78	V66-2821	A	20.11
79	V66-2708	A	20.82
80	V66-2664	A	15.02
81	V66-2566	A	15.44
82	V66-2610	A	20.49
83	V66-2622	A	26.64
84	V66-2731	A	15.60
85	V66-2800	A	18.43
86	V66-2773	A	25.13
87	V66-2730	A	12.55
88	V66-2642	A	19.99
89	V66-2506	A	28.06
90	V66-3001	A	16.85
91	V66-2838	A	26.82

Comments ; Ct = Threshold cycles

APPENDIX B

RESULTS OF INFLUENZA B

Table 1 Results of Influenza B with Solana Influenza A+B assay compared with RT-PCR (No. 1-34).

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
1	V66-0645	B	17.06
2	V66-0714	B	14.29
3	V66-2582	B	26.22
4	V66-2312	B	14.08
5	V66-2536	B	22.71
6	V66-2447	B	18.87
7	V66-2517	B	19.52
8	V66-2539	B	17.91
9	V66-2637	B	16.25
10	V66-2645	B	22.54
11	V66-2304	B	19.89
12	V66-2498	B	20.54
13	V66-2453	B	15.86
14	V66-2451	B	16.92
15	V66-2711	B	16.84
16	V66-2694	B	17.64
17	V66-2553	B	12.88
18	V66-2693	B	20.97
19	V66-2619	B	17.65
20	V66-2303	B	15.43
21	V66-2356	B	17.49
22	V66-2355	B	16.28
23	V66-2327	B	18.68
24	V66-2340	B	16.16
25	V66-2299	B	21.01
26	V66-2659	B	14.66

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
27	V66-2353	B	22.60
28	V66-9624	B	12.58
29	V66-2671	B	15.15
30	V66-2932	B	15.30
31	V66-2981	B	20.04
32	V66-2961	B	16.17
33	V66-2928	B	14.32
34	V66-2906	B	12.80

Comments ; Ct = Threshold cycles

Table 2 Results of Influenza B with Solana Influenza A+B assay compared with RT-PCR (No. 35-68).

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
35	V66-2931	B	18.98
36	V66-2918	B	21.02
37	V66-2975	B	18.20
38	V66-2915	B	18.55
39	V66-2909	B	13.67
40	V66-2999	B	22.36
41	V66-2741	B	24.11
42	V66-2819	B	32.63
43	V66-3015	B	16.11
44	V66-2779	B	18.39
45	V66-3016	B	12.58
46	V66-2682	B	18.65
47	V66-2706	B	17.47
48	V66-3005	B	17.61

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
49	V66-2763	B	21.61
50	V66-2820	B	18.83
51	V66-3011	B	15.83
52	V66-2870	B	12.22
53	V66-2810	B	14.62
54	V66-2862	B	19.05
55	V66-2833	B	13.17
56	V66-2557	B	19.37
57	V66-2716	B	16.35
58	V66-3004	B	16.51
59	V66-2863	B	21.46
60	V66-2850	B	25.91
61	V66-2712	B	14.53
62	V66-2871	B	18.93
63	V66-2823	B	27.27
64	V66-2565	B	16.79
65	V66-2845	B	18.27
66	V66-2818	B	13.35
67	V66-2822	B	14.63
68	V66-2583	B	26.48

Comments ; Ct = Threshold cycles

Table 3 Results of Influenza B with Solana Influenza A+B assay compared with RT-PCR (No.69-99).

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
69	V66-2875	B	20.93
70	V66-2668	B	15.06
71	V66-9681	B	14.41
72	V66-0748	B	21.39
73	V66-9991	B	24.56
74	V66-0104	B	20.81
75	V66-9704	B	19.23
76	V66-9765	B	22.79
77	V66-0814	B	12.19
78	V66-9995	B	19.73
79	V66-9926	B	19.00
80	V66-0083	B	15.04
81	V66-0146	B	15.01
82	V66-0644	B	15.57
83	V66-2575	B	19.05
84	V66-2400	B	17.58
85	V66-3143	B	17.85
86	V66-3114	B	14.85
87	V66-3089	B	14.85
88	V66-2466	B	15.02
89	V66-2439	B	19.41
90	V66-3124	B	13.28
91	V66-3037	B	16.28
92	V66-3059	B	16.23
93	V66-3060	B	16.53
94	V66-3151	B	14.06

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
95	V66-3157	B	13.18
96	V66-3131	B	11.98
97	V66-2836	B	27.21
98	V66-2847	B	22.23
99	V66-2997	B	14.36

Comments ; Ct = Threshold cycles

APPENDIX C

RESULTS OF INFLUENZA NEGATIVE

Table 1 Results of Influenza Negatine with Solana Influenza A+B assay compared with RT-PCR (No.1-34).

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
1	V66-2992	Negative	Negative
2	V66-2816	Negative	Negative
3	V66-2815	Negative	Negative
4	V66-2994	Negative	Negative
5	V66-2829	Negative	Negative
6	V66-2824	Negative	Negative
7	V66-2830	Negative	Negative
8	V66-2848	Negative	Negative
9	V66-2806	Negative	Negative
10	V66-2789	Negative	Negative
11	V66-2843	Negative	Negative
12	V66-2854	Negative	Negative
13	V66-2841	Negative	Negative
14	V66-2998	Negative	Negative
15	V66-3000	Negative	Negative
16	V66-2837	Negative	Negative
17	V66-2990	Negative	Negative
18	V66-2807	Negative	Negative
19	V66-2805	Negative	Negative
20	V66-2844	Negative	Negative
21	V66-2989	Negative	Negative
22	V66-2988	Negative	Negative
23	V66-2842	Negative	Negative
24	V66-2856	Negative	Negative
25	V66-2828	Negative	Negative

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
26	V66-2986	Negative	Negative
27	V66-2858	Negative	Negative
28	V66-2826	Negative	Negative
29	V66-2825	Negative	Negative
30	V66-2857	Negative	Negative
31	V66-2840	Negative	Negative
32	V66-2987	Negative	Negative
33	V66-2860	Negative	Negative
34	V66-2985	Negative	Negative

Table 2 Results of Influenza Negatine with Solana Influenza A+B assay compared with RT-PCR (No.35-50).

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
35	V66-2852	Negative	Negative
36	V66-2996	Negative	Negative
37	V66-0708	Negative	Negative
38	V66-0656	Negative	Negative
39	V66-0548	Negative	Negative
40	V66-0673	Negative	Negative
41	V66-9971	Negative	Negative
42	V66-2972	Negative	Negative
43	V66-2835	Negative	Negative
44	V66-2744	Negative	Negative
45	V66-2785	Negative	Negative
46	V66-0551	Negative	Negative
47	V66-2962	Negative	Negative

No.	Code	Results	
		Solana Influenza A+B assay	RT-PCR (Ct)
48	V66-2736	Negative	Negative
49	V66-9689	Negative	Negative
50	V66-0190	Negative	Negative

APPENDIX D

RESULTS OF INFLUENZA CROSS-REACTION

Table 1 Results of Influenza cross reaction with Solana Influenza A+B assay (No.1-20).

No.	Pathogen list	Results
1	Human rhinoviruses	Negative
2	Human rhinoviruses	Negative
3	Human rhinoviruses	Negative
4	Human rhinoviruses	Negative
5	Human respiratory syncytial viruses A	Negative
6	Human respiratory syncytial viruses A	Negative
7	Human respiratory syncytial viruses A	Negative
8	Human respiratory syncytial viruses B	Negative
9	Human respiratory syncytial viruses B	Negative
10	Human respiratory syncytial viruses B	Negative
11	human coronaviruses 229E	Negative
12	human coronaviruses 229E	Negative
13	human coronaviruses 229E	Negative
14	human coronaviruses 229E	Negative
15	human coronaviruses OC43	Negative
16	human coronaviruses OC43	Negative
17	human coronaviruses OC43	Negative
18	human coronaviruses OC43	Negative
19	Human metapneumoviruses	Negative
20	Human metapneumoviruses	Negative

BIOGRAPHY

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