IDENTIFICATION OF INFLUENZA VIRUS ON FIREFLYDX PROTOTYPE

Discussion

The timely and accurate diagnosis of influenza infection is becoming increasingly important this flu season, which is proving to be a very serious one with a higher than normal number of fatalities and hospitalizations. Being able to accurately diagnose flu "Types" at the point of care empowers health care providers with the information they need for appropriate treatment protocols. It isn't always enough to rely on a rapid influenza detection test result, whether positive or negative. Health care providers need to be armed with the necessary tools to decipher which patients have the flu vs. a common cold, and if the flu is present, it is imperative to know which type is present to ensure the best possible health outcomes, pinpoint geographic areas facing outbreaks, and minimize the spread of infection.

Background

Influenza is an infectious disease caused by an influenza virus and is known commonly as the "flu." Flu symptoms can be mild to severe with the most common symptoms including a high fever, runny nose, sore throat, muscle pains, headache, coughing, and fatigue. These symptoms are also very common with other infectious diseases, so much so that doctors often use the phrase, "flu-like symptoms," which can make it hard to distinguish from other illnesses such as the common cold. This also stresses the need to perform diagnostic testing to be certain that patients with these symptoms do or do not have the flu.

The flu spreads around the world in a yearly outbreak called Seasonal Flu and results in about three to five million cases of severe illness and up to 650,000 deaths annually, according to new estimates by the United States Centers for Disease Control and Prevention (US-CDC), the World Health Organization and global health partners. Roughly three times per century, a flu pandemic occurs. Flu pandemics cause very severe illness and deaths and can infect a large proportion of the world's population with the potential to kill tens of millions of people. The most famous and lethal outbreak was the 1918 flu pandemic, also known as the Spanish flu pandemic, which lasted from 1918 to 1919. It is not known exactly how many people it killed, but estimates range from 50 to 100 million people. It has been described as "the greatest medical holocaust in history" and may have killed as many people as the Black Death.

There are three "types" of influenza viruses that affect people: Type A, Type B, and Type C. The Type A viruses are the most virulent human pathogens among the three influenza types and cause the most severe disease. Wild aquatic birds are the natural hosts for most Type A flu viruses, which occasionally are transmitted to other species (pigs, horses, and birds) and may then cause devastating outbreaks in domestic poultry or give rise to human influenza pandemics as described above. It is important to have accurate diagnostic tests performed to know which type of flu virus a patient has, which results then lead to treatment decisions.

Diagnosing the Flu in Patients

Testing during an outbreak of acute respiratory disease can determine if influenza is the cause and guide prompt implementation of prevention and control measures. Diagnostic tests to confirm flu infections and distinguish between flu types may be performed by testing the throat, sputum, or nose for the virus. A number of rapid tests are available at the doctor's office; however, people may still be infected with the flu, even if the results are negative because of the limited sensitivity and specificity of currently available tests. A type of test called polymerase chain reaction (PCR) that detects the virus' RNA (genetic code) is much more accurate and can give more detailed information about the flu virus, such as what flu type is causing the infection and if the virus may have the potential to be a pandemic strain. However, PCR tests are not used as commonly as rapid tests at the point of care due to the expense, the type of equipment required, the time needed for detection (several hours or more), and the necessary technical expertise for PCR testing.

The winter season that began in 2017 and will end in 2018 "unquestionably falls into a bad year," says Anthony Fauci, director of the National Institutes of Health National Institute of Allergy and Infectious Disease. The flu strain circulating this season is a Type A virus called H3N2 and is very similar to a strain called H1N1 that was responsible for the 1918 flu pandemic mentioned above, as well as the 2009 "swine flu" outbreak. The need is very clear for rapid, sensitive and specific, highly accurate diagnostic testing readily available for healthcare workers to test patients at the point-of-care.

How Well Do Rapid Influenza Detection Tests Work?

During an influenza outbreak, a positive rapid flu test is likely to indicate influenza infection. However, Rapid Influenza Detection Tests (RIDTs) vary in their ability to detect flu viruses depending on the type of rapid test used and the type of flu viruses circulating, particularly when compared with the sensitivity and specificity of viral culture or real-time PCR.

Not all RIDTs can distinguish between influenza A and B, which may represent an important distinction in the severity of the illness, and no RIDTs can distinguish between the varying strains of influenza A. Also, rapid tests appear to be better at detecting flu in children than adults. This variation in ability to detect viruses can result in some people who are infected with the flu having a negative rapid test result. (This situation is called a false negative test result.) Additionally, when compared with viral cell cultures and PCR or other molecular assay testing methods, RIDTs have the lowest sensitivities and the highest rate of false-negatives, meaning they may only detect up to 70% of flu cases.

Despite a negative rapid test result, a health care provider may diagnose a patient with flu based on his or her symptoms and the provider's clinical judgment. Part of the patient's treatment plan could include the prescription of anti-viral medication. The potential downside to prescribing anti-viral medication if the patient does not have the flu is that over-prescription may ultimately result in less effective medicines in the future as viruses become resistant.

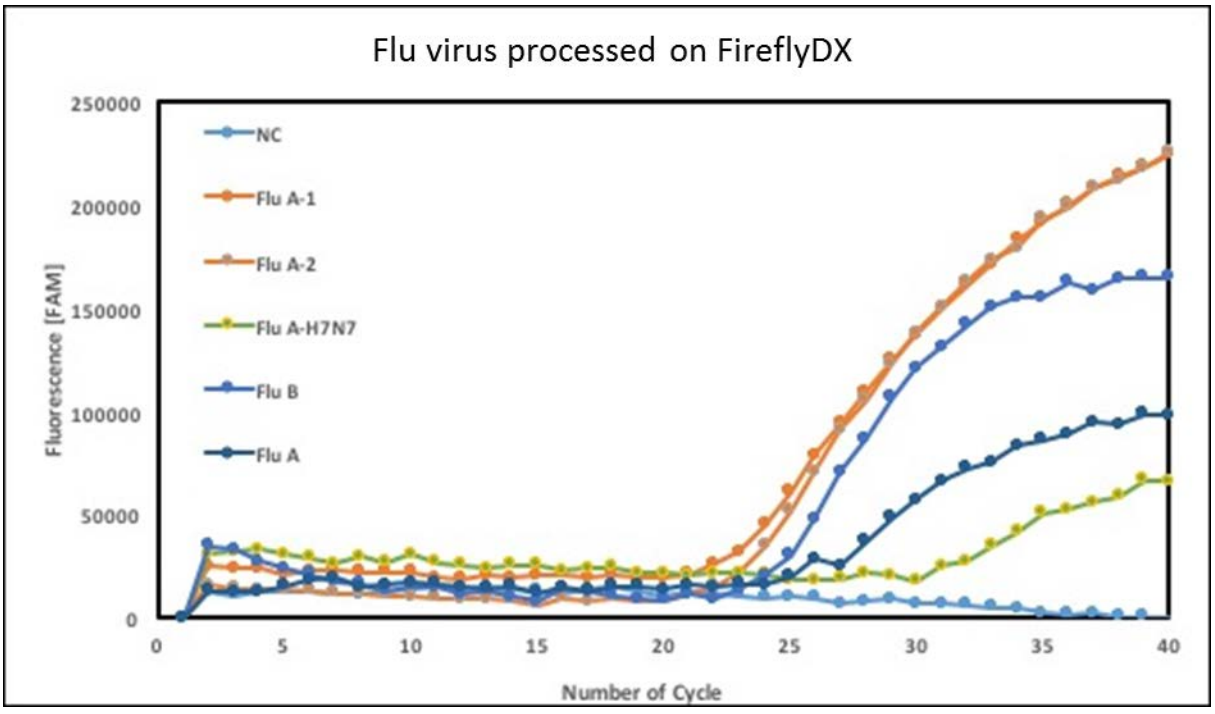
In that regard, if an important clinical decision is affected by a flu test result, the RIDT result should be confirmed by a molecular assay, such as PCR.

FireflyDX

PositiveID and its ExcitePCR subsidiary are developing FireflyDX, in both portable and handheld forms, to offer rapid sample-to-result detection in less than 30 minutes using real-time PCR chemistry. FireflyDX is capable of multiplex assays and designed to utilize lyophilized reagents on a single-use, disposable cartridge for lab-quality results at the point of care or point of need (POC/PON). The system will combine sample lysis, purification, real-time PCR analysis, and wireless reporting of results. The system is designed to process a variety of sample types, including whole blood, buccal and nasopharyngeal swabs, urine, and environmental field samples. PON detection can provide faster results, anywhere and anytime, that are comparable with results delivered in a lab setting. Importantly, using PCR technology to detect flu does not require additional confirmatory testing, unlike RIDTs.

The FireflyDX breadboard prototype system has successfully detected different strains of influenza in the lab. Influenza is an RNA virus and requires a reverse transcription step to create cDNA prior to PCR being performed. The graph below illustrates the flu viruses processed on the FireflyDX. The entire testing process was performed on the FireflyDX breadboard, which is significantly faster than standard laboratory equipment and protocols, with PCR results achieved in minutes instead of hours or days.

Testing Data



Influenza virus was successfully detected on the FireflyDX breadboard prototype pathogen detection system in a collaborative effort with PositiveID's assay testing partner, GenArraytion. Five different flu virus assays (Type A; Type B; H3N3; H7N1; and, H5N1) were tested at low number of copies via real-time

PCR and the data curves crossing threshold at 25 to 27 Ct. The disposable chip was loaded with the reaction and then inserted onto the FireflyDX prototype system. The automated runs successfully synthesized cDNA using a reverse transcriptase (RT) step and then completed a 40-cycle PCR to produce the detected target results.

Multiple samples have been successfully processed on the FireflyDX sample preparation prototype, whereafter the purified sample DNA was put through the PCR process and real-time detection on the FireflyDX PCR prototype, without the use of any commercial instruments. The next step in the development of FireflyDX is to combine these processes and prototypes into a single unit and demonstrate the capability to run a test from putting the raw sample in the cartridge through sample preparation, PCR and real-time detection as a single system.

Summary

Rapid POC/PON tests are imperative to accurately diagnose flu in a timely manner. FireflyDX is designed to detect and identify influenza at the point of need with lab-grade results in less than 30 minutes. This is simply not possible with existing systems which require lab-based equipment, technical personnel, and can take hours or even days to provide results. The low cost, ease of use, and device-to-cloud connectivity may facilitate earlier and more accurate consideration for antiviral drug use and precautions, particularly for at-risk individuals to improve clinical outcomes and maximize pandemic and seasonal control of influenza.

References

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