Simplexa™ RSV & Influenza A/B PCR Background and Principle

BACKGROUND

Infections with influenza A, influenza B and respiratory syncytial viruses (RSV) are major causes of upper and lower tract diseases in infants and young children ranging from mild cold symptoms to life-threatening infections. Individuals that have compromised immune systems are particularly at risk for severe disease with these viruses, especially children <2 years and adults > 65 years of age. Influenza viruses are the cause of "flu" and occasionally croup or pneumonia and have been associated with acute myositis and Reye syndrome. RSV is the most common cause of bronchiolitis and pneumonia in infants and young children and the leading cause of hospitalization during the first 3 months of life. RSV and influenza epidemics occur every year during the winter months. The duration and intensity of the epidemics vary from year to year. Transmission is by airborne droplets (coughing and sneezing) and direct or close contact with contaminated secretions[1, 2, 3].

RSV is a RNA virus in the family *Paramyxoviridae* and is related to human metapneumoviruses and parainfluenza viruses. There are 2 subtypes, RSV A and RSV B that often circulate in the same season. RSV A replicates at higher titers and is thought to be more severe than infections with RSV B[1, 2, 3].

Influenza viruses are RNA viruses in the family *Orthomyxoviridae* and have 3 antigenic types, A, B and C. Annual epidemics are caused by influenza types A and B. Both types A and type B are included in the vaccine. Generally, influenza B causes less severe disease than influenza A. Influenza C causes a mild upper respiratory illness in children and is not included in the vaccine. Influenza A viruses continually undergo genetic changes, that include genetic drift (random mutation) and genetic shift, a reassortment of 2 surface antigens, hemagglutinin (H) and neuraminidase (N) that determine influenza A subtypes. Reassortment refers to a human influenza A subtype acquiring a novel H or N gene segment from a non-human source found in pigs, chickens, ducks, etc. The genetic changes generate new strains every year which impact the efficacy of the vaccine. It is genetic drifts that are responsible for the year-to-year appearance of influenza A. Pandemics, such as the "Spanish flu" in 1918, are rare and a result of a genetic shift[4]. During the flu season, all the types, A, B and C, can circulate with type A being dominant[1, 2, 3].

PRINCIPLE

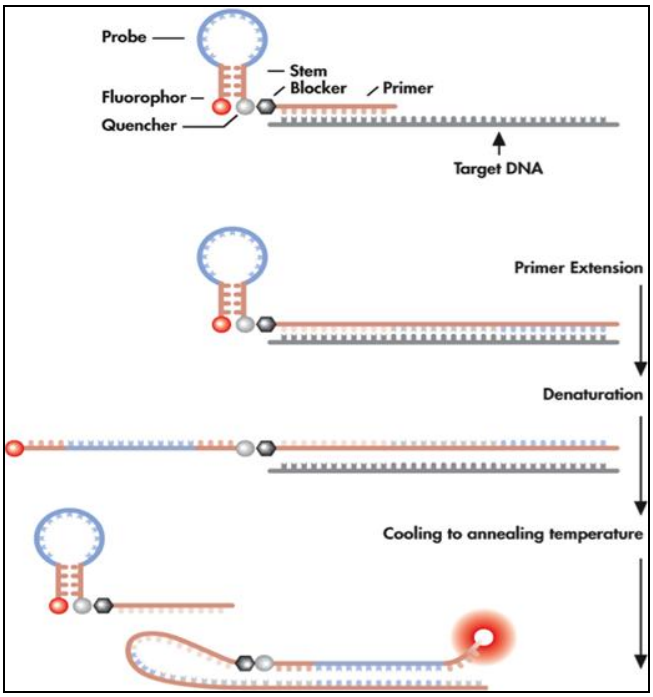
The Simplexa™ Flu A/B & RSV Direct assay uses real time PCR amplification and detection using a scorpion primer-probe with a fluorescent reporter molecule incorporated into a single oligonucleotide The Scorpion primer is located at the 3' end which carries a Scorpion probe contained within the hairpin loop structure at 5' end. The primer sequence contains a PCR blocker at the start of the hairpin loop. The blocker prevents the Taq DNA polymerase from reading through the Scorpion primer and copying the probe region that would lead to the detection of non-specific PCR products. The probe is a self-complementary stem sequence with a fluorophore at one end and a quencher at the other end. The loop of the probe includes a sequence that is complementary to an internal portion of the target sequence. If the intended target is present, the probe hybridizes to the complementary strand separating the reporter molecule from the quencher allowing the reporter to fluoresce. The resulting signal is proportional to the amount of amplified product in the sample[1]. An internal control (IC) is included in the assay that is amplified at the same time to detect PCR inhibition and to confirm that the reagents are working properly.

Since the Scorpion probe and primer are physically linked, the probe reaction kinetics are extremely fast. The unimolecular reaction allows the Scorpion probes to provide stronger signals, shorter reaction times and better discrimination than other conventional bi-molecular techniques[3, 6].

Figure 1: Gene target

Analyte	Gene Targeted	Probe Fluorophore	Excitation	Emission
Influenza A	matrix gene	FAM	495 nm	520 nm
Influenza B	matrix gene	JOE	520 nm	548 nm
Respiratory syncytial virus (RSV)	M gene	CFR610	590 mm	610 mm
Internal control	NA	Q670	644 nm	670 nm

Figure 2: Scorpion Primer Function



1. The Scorpion primer acts as a primer and a probe. The probe forms a hairpin loop with a self-complimentary stem sequence so that the quenched reporter does not fluoresce. The primer is linked to the probe at the start of the hairpin loop.

2. During the annealing, the primer binds to the template and is extended.

3. The probe part of the Scorpion is complementary to the extension product of the attached primer. When the complementary strands are separated in the denaturation step of the next PCR step, the reporter separates from the quencher and opens the loop. When cooled to annealing temperature, the probe sequence binds to the internal target sequence. The reporter and the quencher are now far enough apart to generate detectable fluorescence.

REFERENCES

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Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1	P. Ackerman	11.20.16	Initial Version