Performance evaluation of a rapid multiplex point-of-care microfluidic immunofluorescence assay for the detection of SARS-CoV-2 Antigen and Flu A/B

Introduction

Some symptoms of COVID-19 (fever, cough, myalgia and shortness of breath) overlap with influenza, and therefore accurate, differentiating tests that can be used at the point of care are required to confirm infection and implement correct treatment[1]. A new, rapid point of care test for SARS-CoV-2 & Flu A/B was developed and validated against RT-PCR using single anterior nasal swab samples from symptomatic clinical subjects able to aid in diagnosis and guide appropriate treatment to multiplex test for all 3 virus antigens.

The LumiraDx SARS-CoV-2 & Flu A/B test is an automated, rapid, microfluidic immunofluorescence assay for use with the LumiraDx Platform, for near-patient testing, intended for the qualitative detection and differentiation of SARS-CoV-2, Influenza A and/or Influenza B viral antigens from nasal swab samples. Samples are collected from individuals suspected of respiratory viral infection consistent with COVID-19 by their healthcare provider. The LumiraDx SARS-CoV-2 & Flu A/B test is intended for use as an aid in the differential diagnosis of SARS-CoV-2, Influenza A, and Influenza B in humans and is not intended to detect Influenza C.

The World Health Organisation (WHO) have named the disease caused by SARS-CoV-2 virus as coronavirus 2019 or COVID-19[1]. The most common symptoms of COVID-19 are fever, tiredness, dry cough. Some patients may have aches and pains, nasal congestion, headache, conjunctivitis, sore throat, diarrhea, loss of taste or smell, or a rash on skin or discoloration of fingers or toes. These symptoms are usually mild and begin gradually. Some people become infected but do not develop any symptoms and do not feel unwell. However, the disease can develop rapidly and have high morbidity in certain populations, especially those with underlying health conditions. The disease can spread from person to person through small droplets from the nose or mouth which are spread when a person with COVID-19 coughs or exhales. Most estimates of the incubation period for COVID-19 range from 2-14 days[2]. Influenza (commonly known as 'flu') is a highly contagious, acute viral infection of the respiratory tract. It is a communicable disease easily transmitted through the coughing and sneezing of aerosolized droplets containing live virus. Influenza outbreaks occur each year during the fall and winter months. Type A viruses are typically more prevalent than type B viruses and are associated with most serious Influenza epidemics, while type B infections are usually milder[3]. The use of a LumiraDx SARS-CoV-2 & Flu A/B test will enable the physician to help verify infection quickly, differentiate between SARS-CoV-2 and Influenza infection, begin appropriate treatment and to initiate isolation precautions helping prevent further spread of infection.

Materials

The performance of the LumiraDx SARS-CoV-2 & Flu A/B test was established with anterior nares nasal swabs (Copan FLOQ Swab) prospectively collected from individual subjects up to 12 days since symptom onset for SARS and 4 days for Flu. For SARS-CoV-2, the samples were collected in the US between June and September 2020 during
the SARS-CoV-2 pandemic. Influenza samples were collected in the US between January and March 2020, ahead of the SARS-CoV-2 pandemic. Due to lack of circulating Influenza, frozen samples were used to determine the performance of the LumiraDx SARS-CoV-2 & Flu A/B Test for Influenza.

Samples were collected from sequentially enrolled subjects who presented with symptoms of Influenza A/B (159) or COVID-19 (188). For SARS-CoV-2, dual nasal swabs were simultaneously collected for testing with the LumiraDx test or the reference test (EUA authorized PCR method). For Influenza, dual nasal swabs were randomised and collected for testing with the LumiraDx test or the reference test (510K cleared PCR method). Swabs were collected and extracted into the LumiraDx Extraction Buffer. Samples were frozen within 1h of collection and

stored until tested. Samples were thawed and sequentially tested according to the Product Insert, with operators blinded to the reference test result. The performance of LumiraDx SARS-CoV-2 & Flu A/B test was compared to the results from nasal swabs collected into 3ml universal transport medium (UTM) and tested with the reference methods.

Results

SARS-CoV-2 analysis of symptomatic subjects using the LumiraDx SARS-CoV-2 & Flu A/B Test, up to 12 days since symptom onset demonstrated Positive Percent Agreement (PPA) 95.5% (CI: 84.9 – 98.7%) and Negative Percent Agreement (NPA) 97.0% (CI: 94.5- 98.4%) (Table 1). The laboratory reference method used to determine Ct was the Roche Cobas® 6800 SARS-CoV-2. The ages of the Subjects included ranged from <1 to 90 years of age. In Table 2, this shows the number of positive subjects correctly identified by the LumiraDx device vs RT-PCR across days since symptom onset.

Influenza A demonstrated PPA 83.3% and NPA 97.5% (Table 3) and Influenza B analysis demonstrated PPA 80.0% and NPA 95.3%. (Table 4).

Table 1: Performance for detection of SARS-CoV-2

		Reference RT-PCR Assay			Wilson Score 95% Confidence Interval			
		Positive	**Negative**	**Total**				
SARS-CoV-2 & Flu A/B	Positive	42	9	51	**PPA**	95.5%	84.9%	98.7%
	Negative	2	294	296	**NPA**	97.0%	94.5%	98.4%
	Total	44	303	347	**PPV**	82.4%	69.7%	90.4%
					NPV	99.3%	97.6%	99.8%
					Prevalence	12.7%	9.6%	16.6%
					OPA	96.8%	94.4%	98.2%

The performance of the LumiraDx SARS-CoV-2 & Flu A/B test was compared to the results from nasal swabs collected into 3mL universal transport medium (UTM) and tested with an EUA PCR method.

Table 2. The performance of SARS-CoV-2 Positive subjects identified by the LumiraDx device vs RT-PCR across days since symptom onset:

Days Since Symptom Onset	Cumulative PCR Positive (+)	LumiraDx Positive (+)	Sensitivity (PPA)	LCI	UCI
0	3	3	100.0%	43.9%	100.0%
1	8	8	100.0%	67.6%	100.0%
2	19	19	100.0%	83.2%	100.0%
3	24	24	100.0%	86.2%	100.0%
4	32	31	96.9%	84.3%	99.4%
5	34	33	97.1%	85.1%	99.5%
6	37	36	97.3%	86.2%	99.5%
7	39	38	97.4%	86.8%	99.5%
8	40	38	95.0%	83.5%	98.6%
9	40	38	95.0%	83.5%	98.6%
10	41	39	95.1%	83.9%	98.7%
11	43	41	95.3%	84.5%	98.7%
12	44	42	95.5%	84.9%	98.7%

Table 3: Performance for detection of Flu A

		Reference RT-PCR Assay				Wilson Score 95% Confidence Interval		
		Positive	Negative	Total				
SARS-CoV-2 & Flu A/B	Positive	25	8	33	**PPA**	83.3%	66.4%	92.7%
	Negative	5	309	314	**NPA**	97.5%	95.1%	98.7%
	Total	30	317	347	**PPV**	75.8%	59.0%	87.2%
					NPV	98.4%	96.3%	99.3%
					Prevalence	8.6%	6.1%	12.1%
					OPA	96.3%	93.7%	97.8%

The performance of the LumiraDx SARS-CoV-2 & Flu A/B test was compared to the results from nasal swabs collected into 3mL universal transport medium (UTM) and tested with a 510K PCR method.

Table 4: Performance for detection of Flu B

		Reference RT-PCR Assay			Wilson Score 95% Confidence Interval			
		Positive	Negative	Total				
SARS-CoV-2 & Flu A/B	Positive	24	15	39	**PPA**	80.0%	62.7%	90.5%
	Negative	6	302	308	**NPA**	95.3%	92.3%	97.1%
	Total	30	317	347	**PPV**	61.5%	45.9%	75.1%
					NPV	98.1%	95.8%	99.1%
					Prevalence	8.6%	6.1%	12.1%
					OPA	93.9%	90.9%	96.0%

The performance of the LumiraDx SARS-CoV-2 & Flu A/B test was compared to the results from nasal swabs collected into 3mL universal transport medium (UTM) and tested with a 510K PCR method.

The performance of the LumiraDx SARS-CoV-2 & Flu A/B test was further analysed for SARS-CoV-2 Antigen with anterior nares nasal swabs prospectively collected from individual subjects and study samples combined to create a larger sample set. The samples were collected in the US and UK between June and September 2020 during the SARS-CoV-2 pandemic. Samples were collected from sequentially enrolled subjects. Dual nasal swabs were simultaneously collected for testing with the LumiraDx test or the reference test (RT- PCR method). Swabs were collected and extracted into the LumiraDx Extraction Buffer. Samples were frozen within 1h of collection and stored until tested. Samples were thawed and sequentially tested according to the Product Insert, with operators blinded to the reference test result. The performance of the LumiraDx SARS-CoV-2 & Flu A/B test was compared to the results from nasal swabs collected into 3ml universal transport medium (UTM) and tested with the reference methods.

SARS-CoV-2 analysis of symptomatic subjects up to 7 days since symptom onset demonstrated Positive Percent Agreement (PPA) 97.4% (CI: 86.8 – 99.5%)

Table 5 shows the agreement between the LumiraDx SARS-CoV-2 & Flu A/B test and the Reference RT-PCR assay for Detection of SARS CoV-2.

Table 5. Agreement between the LumiraDx SARS-CoV-2 & Flu A/B test and the Reference RT-PCR assay for Detection of SARS CoV-2.

	Grouping	PCR +ve	LDx +ve	PPA	CI	PCR -ve	LDx -ve	NPA	CI
DSSO	≤ 5	103	95	92.2%	85.4%-96.0%	246	244	99.2%	97.1%-99.8%
	≤ 6	116	107	92.2%	85.9%-95.9%	252	250	99.2%	97.2%-99.8%
	≤ 7	126	115	91.3%	85.0%-95.1%	271	268	98.9%	96.8%-99.6%
	≤ 10	134	120	89.6%	83.2%-93.7%	284	281	98.9%	96.9%-99.6%
	Ct< 33 (all)	122	-	88.5%	81.7%-93.0%				
	Ct< 30 (all)	110	-	91.8%	85.2%-95.6%	DSSO = Days Since Symptom Onset			
	Ct< 25 (all)	64	-	96.9%	89.3%-99.1%				

Analytical performance Limit of Detection – LoD (Analytical sensitivity)

Limit of Detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2, Flu A and/or Flu B at which at least 95% of all (true positive) replicates test positive. The LoD for the LumiraDx SARS-CoV-2 & Flu A/B test was established using limiting dilutions of gamma-irradiated SARS-CoV-2 (BEI Resources NR-52287), Influenza A H1N1 California 07/2009 (BEI Resources VR-1894)), Influenza A H3N2 Perth/16/09 (Zeptometrix 0810251CF), Influenza B Victoria/2/87 (Zeptometrix 0810517CF) and Influenza B Wisconsin/1/10 (Zeptometrix 0810241CF) viruses. The NR-52287 is a preparation of SARS-Related Coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020, that has been inactivated by gamma-irradiation at 5 x 10^6 RADS. The material was supplied frozen at a concentration of 2.8 x 10^5 TCID50/mL. The Influenza viruses are all live viruses and were supplied frozen at concentrations of 4.17 x10^5 (Flu A California/07/2009), 5 x 10^4 (Flu A H3N2 Hong Kong/6/68) 5 x 10^3 (Flu B Brisbane 60/08) and 3.89 x 10^4 (Flu B Wisconsin/1/10) $TCID_{50}$/mL respectively.

Table 6.

Virus Material	Starting Concentration	Estimated LoD	No. Positive/ Total	% Postive
SARS-CoV-2 USA-WA1/2020	2.8 x 10^5 $TCID_{50}$/mL	80 $TCID_{50}$/mL	20/20	100
Flu A H1N1 California/07/2009	4.17 x 10^5 $TCID_{50}$/mL	200 $TCID_{50}$/mL	20/20	100
Flu A H3N2 Hong Kong/6/68	5 x 10^4 $TCID_{50}$/mL	100 $TCID_{50}$/mL	20/20	100
Flu B Brisbane 60/08	5 x 10^3 $TCID_{50}$/mL	100 $TCID_{50}$/mL	20/20	100
Flu B Wisconsin/1/10	3.89 x 10^4 $TCID_{50}$/mL	40 $TCID_{50}$/mL	20/20	100

Conclusion

In clinical studies, the LumiraDx SARS-COV-2 & Flu A/B test demonstrated up to 95.5%, 83.3% and 80.0% positive agreement versus RT-PCR for detection of SARS-COV-2, Flu A and Flu B respectively, using anterior nasal swabs from symptomatic participants.

The LumiraDx SARS-CoV-2 & Flu A/B Test is able to be used to verify potential infection quickly with a rapid microfluidic assay that provides actionable and lab-comparable results in 12 mins for patients suspected of Flu and/or COVID-19, to help decision making and guide treatment. Both SARS-CoV-2 and influenza infections can present with similar symptoms, but treatment decisions are different[4]. Differentiating SARS-CoV-2 from influenza at the point of care allows clinician to optimally guide infection control and treatment decisions.

The LumiraDx SARS-CoV-2 & Flu A/B test results should be evaluated by a Healthcare Professional in the context of all available clinical and laboratory data.

Please refer to the LumiraDx SARS-CoV & Flu A/B Product Insert for more details at **www.lumiradx.com**

References

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The LumiraDx SARS-CoV-2 & Flu A/B has achieved CE Mark.

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