Abstract
Clinical Performance of the LumiraDx SARS-CoV-2 Ag Ultra Test

Introduction

The LumiraDx SARS-CoV-2 Ag Ultra test is an automated rapid microfluidic immunofluorescence assay for use with the LumiraDx Platform, reporting the patient result in under 5 minutes from sample application. The test is intended for near-patient testing and the qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2 directly from anterior nasal swab samples collected from individuals suspected of COVID-19 by their healthcare provider within the first twelve days of symptom onset or from individuals without symptoms or other epidemiological reasons to suspect COVID-19.

Clinical Performance

Multiple datasets have been obtained to determine the clinical performance of the LumiraDx SARS-CoV-2 Ag Ultra test.

Clinical Performance With Samples Collected From Symptomatic Individuals

The performance of the LumiraDx SARS-CoV-2 Ag Ultra test was established with 81 direct nasal swabs prospectively collected from individual subjects during the COVID-19 pandemic. Samples were collected from subjects who presented with symptoms of COVID-19 or Influenza like illness. No positive results were observed from patients without symptoms or beyond 12 days since symptom onset (DSSO). Dual nasal swabs were simultaneously collected and then randomly allocated to testing with the LumiraDx test or an EUA authorized PCR reference method. Swabs were collected and extracted into the LumiraDx Extraction Buffer without transport media. Samples were frozen within 1 hour of collection. Samples were thawed and tested according to LumiraDx instructions. The performance of the LumiraDx SARS-CoV-2 Ag Ultra test was compared to the results from nasal swabs collected into 3ml universal transport medium (UTM) and tested with an EUA authorized PCR reference method.

The table below (Table 1) shows the performance measure, and 95% confidence intervals, as calculated with the Wilson Score method for 81 nasal samples collected up to and including 12 DSSO for the detection of SARS-CoV-2.

Grouping	N	PPA	95% CI
Ct (all)	41	92.7%	(80.6%, 97.5%)
Ct <34 (all)	39	97.4%	(86.8%, 99.5%)
Ct <33 (all)	38	97.4%	(86.5%, 99.5%)
Ct <30 (all)	35	97.1%	(85.5%, 99.5%)
Ct <25 (all)	25	100.0%	(86.7%, 100.0%)

Table 1. Samples with Ct's above 33-34 are generally considered to be non-infectious.[1]
PPA (Positive percent agreement), CI (Confidence interval)

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The following table (Table 2) shows the agreement between LumiraDx SARS-CoV-2 Ag Ultra and the Reference RT PCR assay for detection of SARS-CoV-2 in 79 samples collected to Ct 34 and including 12 DSSO.

	RT-PCR to Ct <34					95% Wilson Score CI		
		POS	NEG	Total	Measure	Estimate	LCI	UCI
LumiraDx SARS-CoV-2 Ag Ultra	POS	38	0	38	PPA	97.4%	86.8%	99.5%
	NEG	1	40	41	NPA	100.0%	91.2%	100.0%
	TOTAL	39	40	79	PPV	100.0%	90.8%	100.0%
					NPV	97.6%	87.4%	99.6%
					Prevalence	49.4%	38.6%	60.2%
					OPA (% Agreement)	98.7%	93.2%	99.8%

Table 2. NPA (Negative percent agreement) , PPV (Positive predictive value), NPV (Negative predictive value), OPA (Overall Percent Agreement), LCI (Lower confidence interval), UCI (Upper confidence interval)

Clinical Performance With Samples Collected From Asymptomatic Individuals

Performance of the LumiraDx SARS-CoV-2 Ag Ultra test was further established with 52 anterior nasal swabs collected from asymptomatic subjects. Swabs were collected and extracted into the LumiraDx Extraction Buffer. Samples were frozen within 1 hour of collection and stored until tested. The performance of the LumiraDx SARS CoV-2 Ultra test was compared to the results from paired anterior nasal swab samples collected into 3ml universal transport medium (UTM) and tested with an EUA authorized PCR method.

Table 3, below, shows the performance measure and 95% confidence intervals, as calculated with the Wilson Score method.

Grouping	N	PPA	95% CI
Ct (all)	23	95.7%	(79.0%, 99.2%)
Ct <30 (all)	22	100.0%	(85.1%, 100.0%)
Ct <25 (all)	18	100.0%	(82.4%, 100.0%)

Table 3

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The following table (Table 4) shows the agreement between LumiraDx SARS-CoV-2 Ag Ultra and the Reference RT-PCR assay for detection of SARS-CoV-2 in samples collected from asymptomatic individuals.

	RT-PCR					95% Wilson Score CI		
		POS	NEG	Total	Measure	Estimate	LCI	UCI
LumiraDx SARS-CoV-2 Ag Ultra	POS	22	0	22	PPA	95.7%	79.0%	99.2%
	NEG	1	29	30	NPA	100.0%	88.3%	100.0%
	TOTAL	23	39	52	PPV	100.0%	85.1%	100.0%
					NPV	96.7%	83.3%	99.4%
					Prevalence	44.2%	31.6%	57.7%
					OPA (% Agreement)	98.1%	89.9%	99.7%

Table 4

Clinical Performance - Expanded data set with Anterior Nasal swab as reference method

The performance of the LumiraDx SARS-CoV-2 Ag Ultra test was further investigated using additional samples to create a dataset of 477 direct nasal swabs prospectively collected from individual subjects during the COVID-19 pandemic. Samples were collected from sequentially enrolled subjects who presented with symptoms of COVID-19 or from asymptomatic screening. No positive results were observed from patients who presented with symptoms beyond 12 days since symptom onset (DSSO). The performance of the LumiraDx SARS-CoV-2 Ag Ultra test was compared to the results from nasal swabs collected into 3ml universal transport medium (UTM) and tested with an EUA authorized PCR method.

The following table (Table 5) displays the number of positive and negative subjects correctly identified by the LumiraDx device vs RT-PCR across days since symptom onset (DSSO):

DSSO	Cumulative PCR +ve	LDx +ve	PPA	LCI	UCI	Cumilative PCR -ve	LDx -ve	NPA	LCI	UCI
0	3	3	100.0%	43.9%	100.0%	8	8	100.0%	67.6%	100.0%
4	109	97	89.0%	81.7%	93.6%	238	237	99.6%	97.7%	99.9%
7	138	122	88.4%	82.0%	92.7%	279	278	99.6%	98.0%	99.9%
12	143	127	88.8%	82.6%	93.0%	282	281	99.6%	98.0%	99.9%

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The table below (Table 6) shows the performance measure, and 95% confidence intervals, as calculated with the Wilson Score method for groupings of the results below.

Grouping	N	PPA	95% CI
Ct (all)	166	89.8%	(84.2%, 93.5%)
Ct <35 (all)	149	96.0%	(91.5%, 98.1%)
Ct <34 (all)	144	98.6%	(95.1%, 99.6%)
Ct <33 (all)	141	98.6%	(95.0%, 99.6%)
Ct <30 (all)	128	98.4%	(94.5%, 99.6%)
Ct <25 (all)	91	98.9%	(94.0%, 99.8%)

Table 6

The following table (Table 7) shows the performance measure, and 95% confidence intervals, as calculated with the Wilson Score method for subjects results above.

	RT-PCR					95% Wilson Score CI		
LumiraDx SARS-CoV-2 Ag Ultra		POS	NEG	Total	Measure	Estimate	LCI	UCI
	POS	149	1	150	PPA	89.8%	84.2%	93.5%
	NEG	17	310	327	NPA	99.7%	98.2%	99.9%
	TOTAL	166	311	477	PPV	99.3%	96.3%	99.9%
					NPV	94.8%	91.8%	96.7%
					Prevalence	34.8%	30.7%	39.2%
					OPA (% Agreement)	96.2%	94.1%	97.6%

Table 7

Clinical Performance - Expanded data set with Nasopharyngeal swab as reference method

For 346 subjects in the previous dataset, an additional Nasopharyngeal swab was collected following dual nasal collection. The Nasopharyngeal swab was placed into 3ml universal transport medium (UTM) and tested with an EUA authorized PCR method.

The table below (Table 8), shows the number of positive and negative subjects correctly identified by the LumiraDx device vs RT-PCR across days since symptom onset (DSSO).

DSSO	Cumulative PCR +ve	LDx +ve	PPA	LCI	UCI	Cumilative PCR -ve	LDx -ve	NPA	LCI	UCI
0	3	3	100.0%	43.9%	100.0%	8	8	100.0%	67.6%	100.0%
4	79	69	87.3%	78.2%	93.0%	203	202	99.5%	97.3%	99.9%
7	100	86	86.0%	77.9%	91.5%	241	240	99.6%	97.7%	99.9%
12	103	89	86.4%	78.5%	91.7%	243	242	99.6%	97.7%	99.9%

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The following table (Table 9) shows the performance measure, and 95% confidence intervals, as calculated with the Wilson Score method for groupings of the results below.

Grouping	N	PPA	95% CI
Ct (all)	103	86.4%	(78.5%, 91.7%)
Ct <35 (all)	97	90.7%	(83.3%, 95.0%)
Ct <34 (all)	94	92.6%	(85.4%, 96.3%)
Ct <33 (all)	91	93.4%	(86.4%, 96.9%)
Ct <30 (all)	84	96.4%	(90.0%, 98.8%)
Ct <25 (all)	59	98.3%	(91.0%, 99.7%)

Table 9

The following table (Table 10) shows the performance measure, and 95% confidence intervals, as calculated with the Wilson Score method for the results above, up to and including 12 DSSO using an EUA authorized RT-PCR method as the reference.

	RT-PCR					95% Wilson Score CI		
		POS	NEG	Total	Measure	Estimate	LCI	UCI
LumiraDx SARS-CoV-2 Ag Ultra	POS	89	1	90	PPA	86.4%	78.5%	91.7%
	NEG	14	242	256	NPA	99.6%	97.7%	99.9%
	TOTAL	103	243	346	PPV	98.9%	94.0%	99.8%
					NPV	94.5%	91.0%	96.7%
					Prevalence	29.8%	25.2%	34.8%
					OPA (% Agreement)	95.7%	93.0%	97.4%

Table 10

Analytical Performance (LoD)

Limit of Detection (LoD) studies were carried out to determine the lowest detectable concentration of SARS-CoV-2 at which 95% of all (true positive) replicates test positive. The LoD for the LumiraDx SARS-CoV-2 Ag Ultra test was established using limiting dilutions of Ultraviolet (UV) inactivated SARS-CoV-2 (Zeptometrix 0810622UV).

An initial LoD screening study was performed using a 5-fold serial dilutions of the UV inactivated virus made in pooled negative human nasal matrix starting at a test concentration of 1.6×10^3 $TCID_{50}$/mL and processed for each study. These dilutions were tested in triplicate and across 3 LumiraDx SARS-CoV-2 Ag Ultra Lot numbers. The lowest concentration at which all replicates were positive was chosen for LoD Range finding, which gave a result of 1600 $TCID_{50}$/mL.

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Using the 1600 $TCID_{50}$/mL concentration, the LoD was further refined using a 2-fold dilution series of the UV inactivated virus made in pooled negative human nasal matrix. These dilutions were tested in triplicate. The lowest concentration at which all replicates were positive was treated as the tentative LoD for the LumiraDx SARS-CoV-2 Ag Ultra test. This was 800 $TCID_{50}$/mL. The LoD of the LumiraDx SARS-CoV-2 Ag Ultra test was then confirmed by testing 20 replicates with concentrations at the tentative Limit of Detection. The final LoD of the LumiraDx SARS-CoV-2 Ag Ultra test was determined to be the lowest concentration resulting in positive detection of at least 19 out of 20 replicates. Based on this testing the LoD for nasal swab samples was confirmed as 800 $TCID_{50}$/mL.

For comparability, the LumiraDx 12 minute SARS-CoV-2 Ag test was tested using the UV inactivated virus stock to compare LoD. The results in the table (Table 11) below demonstrate that both test strips have an LoD of 800 $TCID_{50}$/mL. This confirms that the LumiraDx SARS-CoV-2 Ag Ultra test has an equivalent LoD to the LumiraDx SARS-CoV-2 Ag test.

	LumiraDx SARS-CoV-2 Ag test (12 min test)	SARS-CoV-2 Ag Ultra Lot A	SARS-CoV-2 Ag Ultra Lot B
SARS-CoV-2 tested ($TCDI_{50}$/mL using Zeptometrix 0810622UV	Test Result	Test Result	Test Result
600	3/3 positive	3/3 positive	3/3 positive
800	3/3 positive	3/3 positive	3/3 positive
400	0/3 positive	2/3 positive	4/20 positive
200	0/3 positive	0/3 positive	0/3 positive
100	0/3 positive	0/3 positive	0/3 positive
50	0/3 positive	0/3 positive	0/3 positive

Table 11

Conclusion

The LumiraDx SARS-CoV-2 Ag Ultra test can provide accurate testing of more patients, optimization of clinic workflows and assistance in triaging patients without delay by providing results in just 5 minutes. The LumiraDx SARS-CoV-2 Ag Ultra test displays high sensitivity in patients with a Ct value <34 within 12 days of the onset of symptoms, enabling the rapid identification of potentially infectious individuals. In clinical studies, the LumiraDx SARS-CoV-2 Ag Ultra test demonstrated 95.7% positive agreement versus RT-PCR in samples collected from asymptomatic individuals. The high sensitivity of the LumiraDx SARS-CoV-2 Ag Ultra Test in asymptomatic patients can be used as an important tool in breaking the chain of transmission of SARS-CoV-2.

References

1. La Scola B., Le Bideau M., Andreani J., Hoang V.T., Grimaldier C., Colson P. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. Eur J Clin Microbiol Infect Dis. 2020:39(6): 1059-1061