Department of Health & Social Care

Technical Validation for LumiraDx SARS-CoV-2 Ag test

Published: February 2021 Version 1.1

Contents

Assay Description 2

Type of sample to be used in validation 4

Equipment and reagents 5

Performance characteristics 6

Analytical specificity (Interferences and cross-reactions) 8

Diagnostic sensitivity and specificity (Clinical validation with confirmed positives and negatives) 10

Summary 11

Assay Description

1. Intended use: The LumiraDx SARS-CoV-2 Ag test is a rapid microfluidic immunofluorescence assay providing qualitative detection of the nucleocapsid protein antigen to SARS-CoV-2 in symptomatic patients within 12 days of symptom onset from a nasal swab.

   Please note the IFU and CE mark has changed for this device since this validation to include testing of asymptomatic individuals and the use of nasopharyngeal swab specimens.

   The LumiraDx SARS-CoV-2 Ag test does not differentiate between SARS-CoV and SARS-CoV-2. Results are for the identification of SARS-CoV-2 nucleocapsid protein antigen. Antigen is generally detectable in nasal swab specimens during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine the infection status.

   Positive results indicate the presence of viral antigens from infective virus, but clinical correlation with individual's history and other diagnostic information is necessary to confirm the infection status.

   Negative results do not rule out SARS-CoV-2 infection and should be considered in the context of an individual's recent exposures, history and presence of clinical signs and symptoms consistent with COVID-19.

   Results should not be used as the sole basis for treatment or case management decisions, including infection control decisions.

   The LumiraDx SARS-CoV-2 Ag Test is intended for use by individuals trained in point of care settings and proficient in performing tests using the LumiraDx Platform.

   Biosafety: The SARS-CoV-2 inactivation testing: interim report HCM/CoV2/045/v2 LumiraDx from PHE can be found at https://www.gov.uk/government/publications/covid-19-phe-laboratory-assessments-of-inactivation-methods. In the absence of evidence of viral inactivation, the test should be used with appropriate PPE.

2. The LumiraDx Severe Acute Respiratory Syndrome (SARS) CoV-2 Antigen (Ag) Test Strips (hereafter referred to as Test Strips) are to be used with the LumiraDx Platform. The LumiraDx Platform is a point of care system for professional use only, which is used for in vitro diagnostic tests. It comprises of a portable LumiraDx Instrument and a LumiraDx Test Strip. This test is for HEALTHCARE PROFESSIONAL USE ONLY

and allows users to perform tests using small sample volumes and to view results quickly on the Instrument touchscreen.

Type of sample to be used in validation

1. Symptomatic patients were tested within 12 days of symptom onset. Nasal swabs were placed directly into the LumiraDx buffer and processed within 5 hours (if these were unable to be processed within 5 hours, the buffer was stored at -80C and tested within 5 days). All samples were tested as per the IFU.

   Samples were tested at two sites, Leeds and Preston, with additional data provided via the Falcon study. Required numbers 150 positive/250 negative, split between all sites. In addition, samples were collected using the same methodology at three sites in Scotland.

   Standard materials from the National Institute for Biological Standards and Control (NIBSC) were also tested at Leeds to assess the dynamic range of the assay.

Table 1. standard materials from NIBSC; whole virus inactivated by acetic acid-heat treatment (bespoke non-catalogue) in phosphate buffered saline; recombinant protein (bespoke, non-catalogue)

Inactivated virus	10^6/ml
Inactivated virus	10^5/ml
Inactivated virus	10^4/ml
Inactivated virus	10^3/ml
Negative control	Negative control
Recombinant protein	Weak positive
Recombinant protein	Weak positive
Recombinant protein	Positive
Recombinant protein	Positive
Recombinant protein	Negative control

Equipment and reagents

Product Components

- LumiraDx Instrument
- LumiraDx SARS-CoV-2 Ag test strips
- Extraction Buffer vials
- Dropper caps
- Positive and negative control materials should be run when a new lot no. of strips is used, different user, troubleshooting

Testing Capacity

- Number of samples: 1 sample per run, per instrument
- Sample input volume: 30µl per reaction
- Test time: 12 minutes

Validated Swab Types

- Copan Nasal FLOQswab™ Regular
- Puritan HydraFlock™ Sterile Standard Flock Swab
- Aspen Surgical™ Polyester Swab
- SteriPack™ Sterile Polyester Spun Swab
- mwe medical wire Dryswab™ Rayon Swab
- Kang Jian™ Virus Collection Swab

Performance characteristics

Sensitivity and Linearity

Table 2. results from standard materials from NIBSC; whole virus inactivated by acetic acid-heat treatment (bespoke non-catalogue) in phosphate buffered saline; recombinant protein (bespoke, non-catalogue

Standard	Swab 1	Swab 2	Swab 3	Swab 4 replicate 1	Swab 4 replicate 2	Swab 4 replicate 3
$10x^6$copies/ml	Positive	Positive	Positive	Positive	Positive	Positive
$10x^5$copies/ml	Negative	Negative	Negative	Negative	Negative	Negative
$10x^4$copies/ml	Negative	Negative	Negative	Negative	Negative	Negative
$10x^3$copies/ml	Negative	Negative	Negative	Negative	Negative	Negative
Negative Control	Negative	Negative	Negative	Negative	Negative	Negative

Table 3. further dilutions of standard materials to find approximate limit of detection

	Neat	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64	1 in 128
approximate copies/ml	1000000	500000	250000	125000	62500	31250	15625	7812.5
Swab 1	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Negative
Swab 2	Positive	Positive	Positive	Positive	Positive	Negative	Negative	Negative
Swab 3	Positive	Positive	Positive	Positive	Positive	Negative	Negative	Negative

Limits of Detection

The limit of detection was provided in TCID50 /ml by the company (32 TCID50/ml) using BEI Resources gamma-irradiated SARS-CoV-2 (USA WA1/2020) prod# NR-52287 viral

preparation and methodology, as recommended by FDA; however, to enable comparison to TPP, the limit of detection to whole cell virus with copies/ml was required.

The standard material was sourced from NIBSC, produced according to PHE protocols (appendix 1).

The approximate LLOD was calculated as ~62500copies/ml.

NOTE: The materials used for LLOD were those that were available at the time of validation. These were different to those used by the manufacturer. It is known that alternative materials may produce different LLODs.

Analytical specificity (Interferences and cross-reactions)

1. Cross-reactivity to non-target samples/organisms. A range of samples either direct clinical samples or spiked samples that are known positives for other diseases, both closely related (i.e., other coronaviruses), syndromic diseases (i.e., other respiratory viruses and bacteria) and common diseases (i.e. HIV, HBV, HCV, VZV, EBV, CMV) should be tested.

Table 4. Cross reactivity - Data provided by the company

Microorganism	**Source**	**Concentration**	**Cross-reactivity (Yes/No)**	**Interference (Yes/No)**
Human coronavirus 229E	Zeptometrix	1 x 105 PFU/mL	No (3/3 negative)	No (3/3 positive)
Human coronavirus OC43	Zeptometrix	1 x 105 PFU/mL	No (3/3 negative)	No (19/20 positive)
Human coronavirus NL63	Zeptometrix	9.87 x 103 PFU/mL	No (3/3 negative)	No (3/3 positive)
MERS Coronavirus	Zeptometrix	7930 PFU/mL	No (2/2 negative)	No (3/3 positive)
Adenovirus (e.g. C1 Ad. 71)	Zeptometrix	1 x 105 PFU/mL	No (3/3 negative)	No (3/3 positive)
Human Metapneu-movirus (hMPV)	Zeptometrix	1 x 105 PFU/mL	No (3/3 negative)	No (3/3 positive)
Parainfluenza virus Type 1	Zeptometrix	1 x 105 PFU/mL	No (3/3 negative)	No (3/3 positive)
Parainfluenza virus Type 2	Zeptometrix	1 x 105 PFU/mL	No (3/3 negative)	No (3/3 positive)
Parainfluenza virus Type 3	Zeptometrix	1 x 105 PFU/mL	No (3/3 negative)	No (3/3 positive)
Parainfluenza virus Type 4a	Zeptometrix	1 x 105 PFU/mL	No (3/3 negative)	No (3/3 positive)
Influenza A H3N2 (Wisconsin/67/05)	Zeptometrix	8.82 x 104 PFU/mL	No (3/3 negative)	No (3/3 positive)
Influenza A H1N1	Zeptometrix	1 x 105 PFU/mL	No (3/3 negative)	No (3/3 positive)
Influenza B (Malaysia/2506/04)	Zeptometrix	2.92 x 104 PFU/mL	No (3/3 negative)	No (19/20 positive)
Enterovirus	Zeptometrix	1 x 105 PFU/mL	No (3/3 negative)	No (3/3 positive)
Respiratory syncytial virus	Zeptometrix	1 x 105 PFU/mL	No (3/3 negative)	No (3/3 positive)

Rhinovirus	Zeptometrix	4.17 x 105 PFU/mL	No (3/3 negative)	No (3/3 positive)
Haemophilus influenzae	Zeptometrix	1 x 106 CFU/mL	No (3/3 negative)	No (3/3 positive)
Streptococcus pneumoniae	Zeptometrix	1 x 106 CFU/mL	No (3/3 negative)	No (3/3 positive)
Streptococcus pyogenes	Zeptometrix	1 x 106 CFU/mL	No (3/3 negative)	No (3/3 positive)
Candida albicans	Zeptometrix	1 x 106 CFU/mL	No (3/3 negative)	No (3/3 positive)
Pooled human nasal wash	LumiraDx	14% v/v	No (3/3 negative)	No (3/3 positive)
Bordetella pertussis	Zeptometrix	1 x 106 CFU/mL	No (3/3 negative)	No (3/3 positive)
Mycoplasma pneumoniae	ATCC	1 x 106 CFU/mL	No (3/3 negative)	No (3/3 positive)
Chlamydia pneumoniae	ATCC	1 x 106 CFU/mL	No (3/3 negative)	No (3/3 positive)
Legionella pneumophila	Zeptometrix	1 x 106 CFU/mL	No (3/3 negative)	No (3/3 positive)
Mycobacterium tuberculosis	Zeptometrix	1 x 106 CFU/mL	No (3/3 negative)	No (3/3 positive)
Pneumocystis jirovecii	Zeptometrix	1 x 106 CFU/mL	No (3/3 negative)	No (3/3 positive)
Pseudomonas Aeruginosa	Zeptometrix	1 x 106 CFU/mL	No (3/3 negative)	No (3/3 positive)
Staphylococcus Epidermidis	Zeptometrix	1 x 106 CFU/mL	No (3/3 negative)	No (3/3 positive)
Streptococcus Salivarius	Zeptometrix	1 x 106 CFU/mL	No (3/3 negative)	No (3/3 positive)

Diagnostic sensitivity and specificity (Clinical validation with confirmed positives and negatives)

Diagnostic sensitivity. Confirmed clinical samples from patients (positive PCR result) should be used. Preferably, depending on the availability of samples, ~150 samples should be included to align with MHRA TPP. Clinical sensitivity (95% CI) and positive predictive value (PPV) should be calculated in comparison with a CE reference method that itself has good sensitivity and specificity.

Table 5. Comparison with reference method (note prevalence of 20.9)

		Comparator	**Comparator**	
		Positive	Negative	Total
LumiraDx	Positive	119	7	126
LumiraDX	Negative	23	530	553
	Total	142	537	679

Sensitivity = 83.8 (95% CI 76.4-89.2%). This meets the acceptable criteria for sensitivity of the POC TPP (desirable>97% = acceptable = >80%).

Table 6. CT range for samples and sensitivity

CT value range	**Positive on Lumira/positive on comparator**	**Sensitivity (%)**
≤25 (low)	30/31	96.8
25<30 (medium)	6/8	75.0%
≤30	36/39	92.3%
≥30 (high)	5/20	25%

Specificity = 98.7% (95% CI 97.2-99.4%). This meets the acceptable criteria for specificity of the POC TPP (desirable>99% = acceptable = >95%).

Summary

1. TVG uses a wide range of sites in order to validate new technologies/tests. These independent sites use a range of RT-qPCR assays against different genomic regions. It is recognised that for some assay comparisons the sensitivity of RT-qPCR assay(s) may subtly differ from the true sensitivity of the test when compared with the same genomic region. It is also recognised that the CT values of different RT-qPCR assays may differ around 2-3 points with the same amount of virus present, leading to variability in sensitivity obtained in some evaluations using different CT ranges.

2. This test meets the acceptable criteria for sensitivity and specificity designated in the POC TPP.

Published to GOV.UK in pdf format only.

www.gov.uk/dhsc