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Accurate and Rapid Antigen Assay for COVID-19 Diagnostics Using Saliva

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Abstract

The global outbreak of COVID-19 highlighted the need for rapid and accurate diagnostic testing to control the spread of this highly contagious disease [1-5]. Here, we describe the nCoVega COVID-19 antigen rapid test (~ 15min) that can detect the presence of the SARS-COV-2 virus particles from saliva sample on a portable device. The portable reader instrument, the Vega-200, has a small footprint and is designed for use at point of care settings. The test detects the fluorescence signal using wide-field illumination from antigen-antibody complexes captured on a special filter matrix ([6]. Results of this clinical evaluation of 183 subjects demonstrates that the nCoVega COVID-19 test performs at par with qRT-PCR tests [7] (gold standard) for both symptomatic and asymptomatic patients with a strong inverse correlation between RFU (relative fluorescence units) and Ct counts (from RT-PCR). The test has an analytical performance of 15.3 TCID50/mL, and 100% specificity for COVID-19 as compared to other human respiratory viruses, including other human coronaviruses. The working principle of this assay and test system can be used for developing other rapid, inexpensive antigen assays and it can offer an end-to-end, point-of-care solution to meet the continuous demand in tackling existing and emerging infectious diseases across the globe.

Keywords: Antigen; COVID-19; immuno-fluorescence; Point of care; SARS-COV-2; Saliva

Introduction

The nCoVega COVID-19 Antigen test, developed by Kaya 17, Inc., is an antigen-capture assay intended for the qualitative detection of spike protein antigen from SARS-CoV-2 in saliva from individuals, as viral antigen is generally detectable in saliva during the acute phase of infection. Positive results indicate the presence of viral antigens. Our antigen test can be performed by trained non-laboratory or laboratory technicians for point of care use or for general use in clinical labs and hospital settings. The test works on a simple portable instrument called Vega-200 using a custom cartridge, assay reagents, and Vega software. The workflow uses a QR code reader and printer for tracking patient information. The vast majority of COVID-19 antigen tests utilize lateral flow technology, which has inherent limitations in terms of dynamic range, sensitivity and specificity [8-12]. The current rapid antigen tests are approved for nasal or NP swab. Other approaches include magnetic force assisted ELISA, microfluidic ELISA and digital chromatographic immunoassays, all of which require costly and complex custom manufacturing. The nCoVega test utilizes selective antigen-antibody complex capture via filtration and wide field fluorescence imaging on an inexpensive and easy to manufacture disposable cartridge, resulting in sensitivity and specificity

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comparable to the qRT-PCR based gold standard (ref). The developed tests therefore can be easily deployed for large-scale population testing under community health settings, hospitals, and at the point of care with minimal operator training. Our test, which is both rapid and inexpensive, can help with COVID-19 diagnosis and controlling the spread of the global pandemic. The test can also be used to support decisions on infection control strategies such as detecting and isolating asymptomatic cases that could spread the virus as we return to normal lifestyles. The novel nCoVega COVID19 Antigen test is a direct dual probe antigen capture test using two fluorescently labeled primary antibodies (S1 and S2 spike protein recombinant IgG) that bind to SARS-CoV-2 spike protein on viral particles present in a saliva sample.

The workflow for our end-to-end solution including the test and the system is shown below in figure 1.

Materials and Methods

Sample collection and processing: A saliva sample (100ul or more) is collected at the point of care testing by directly spitting into a vial that is placed in a syringe fitted with a filter. 1mL of the proprietary sample processing reagent is added. A plunger is pushed to allow the treated saliva sample to pass through the filter and is collected in a second tube for antibody labeling. The filtrate is incubated with 30ul of fluorescently-labeled S1 and S2 antibodies for 10 minutes at room temperature. The mixture is then passed through a disposable vertical flow cartridge fitted with a proprietary capture matrix. The fluorescence signal from the captured antigen-antibody complex is recorded using a portable device equipped with wide field illumination. Antibodies and conjugation: The S1 and S2 antibodies used in our test are purchased from GeneTex, TX and conjugated with a Biotium Mix-n-Stain CF 405L fluorophore (Biotium Inc, Fremont, CA, USA). The fluorophore used in our test has a large Stokes Shift with an excitation peak at 395nm and emission peak around 545 nm. S1 and S2 antibodies are conjugated using the Mix-n-Stain protocol following the manufacturer's protocol, which consists of direct labeling of the antibodies to the fluorophore via an NHS-ester covalent bond formation. The conjugated antibodies are then purified via spin column centrifugation and buffer exchanged into a PBS-based storage buffer. The CF dyes were carefully chosen for their superior brightness, photostability, large Stokes Shift, and resistance to hydrolysis offering superior performance over most dyes. Instrument and data collection: The patient saliva sample labeled with fluorescent antibodies is placed on the cartridge in a well lined at the bottom with a capture matrix. An absorbent pad inside the cartridge holder to remove excess liquid and the unbound antibody. The bound antibody/antigen complex is selectively retained on the capture matrix via size exclusion mechanism. 500ul of wash buffer is added to the cartridge and allowed to wick through. Finally, 500ul of proprietary read buffer is added to the cartridge and the cartridge is immediately placed on the Vega-200 instrument for readout. The Vega-200 instrument records the fluorescence from the captured complex on the cartridge and reports a qualitative result (Positive or Negative). The system portable Vega-200 instrument is a fluorescence reader (U.S. Patent Application Serial Number: 16/690,589). The Vega analysis software, Patient ID software, an ID scanner with a QR code printer were all run on a standard PC. In addition, data reporting via a mobile device is also available.

Data Availability

Detailed data directly used to generate each figure or table of this study are available within the article, Supplementary Information and source data are also provided with this paper.

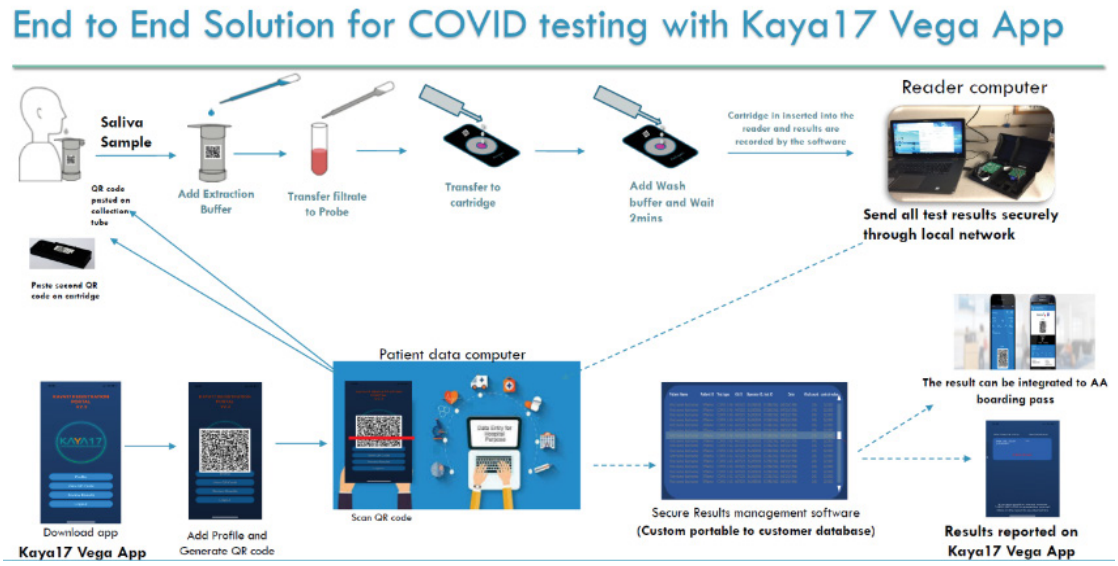

Figure 1: Schematic diagram showing the workflow for the nCoVega Test on the Vega-200.

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Method used to Determine LoD

Concentrations ranging from 1.6 x 105 TCID50/mL to 1.0 x 101 TCID50/mL Heat killed Zeptometrix SARS-CoV-2 (Cat. No. 0810587CFHI Lot No.324930) was spiked into artificial saliva matrix from Pickering laboratory (1700-0316). Limit of Detection (LoD) for Kaya17 nCoVega test was determined by performing serial dilution (Table 1). A 10-fold serial dilution of was performed from 10,000, 1,000, 100, 10 and 0 of Heat Killed SARS CoV 2 from Zeptometrix in triplicate in Artificial saliva matrix from Pickering laboratory (1700-0316) (Figure 2a). From this, the LoD was estimated as 15.3 TCID50 per ml by Probit analysis (Figure 2b). Raw data is included in the supplementary material section (Table 1, supplementary materials section).

Cross Reactivity (Analytical Specificity)

Each organism was tested in the absence or presence of Heat Killed SARS-CoV-2 from Zeptometrix at 3 x LoD in Artificial saliva matrix from Pickering laboratory (1700-0316). The final concentration of the organisms is in the Table 3 below (the concentrations of 106 CFU/mL or higher for bacteria and 105 PFU/mL or higher for viruses is recommended). For some of the microorganisms, the stock concentration was lower than or equal to the recommended testing concentration. In such cases, these microorganisms were used at the stock concentration.

Microbial Interference Studies

Each organism was tested in the absence or presence of Heat Killed SARS-CoV-2 from Zeptometrix at 3 x LoD in Artificial saliva matrix from Pickering laboratory (1700-0316). The final concentration of the organisms is in the Table 4 below (the concentrations of 10^6 CFU/mL or higher for bacteria and 10^5 PFU/mL or higher for viruses is recommended). For some of the microorganisms, the stock concentration was lower than or equal to the recommended testing concentration. In such cases, these microorganisms were used at the stock concentration.

Endogenous Interference Studies

Each substance was tested in triplicate in the absence or presence of Heat Killed SARS-CoV-2 from Zeptometrix at 3 x LOD in Artificial saliva matrix from Pickering laboratory (1700-0316). Substances for testing were selected based on the respiratory specimen's guidance in http://www.accessdata.fda.gov/cdrh_docs/reviews/K112177.pdf.

Hook Effect Testing

To determine if the Kaya17 nCoVega Test suffers from any high dose Hook effect, increasing concentrations of Heat Killed SARS-CoV-2 from Zeptometrix at 3 x LoD, were tested up to a concentration of 1.6 x 105 TCID50/mL. The starting material was spiked into a pooled negative saliva

Table 1: LOD LOB testing raw data.

ID	Bgd_ Rdg	Thr_Rdg	Raw_Avg	Test_ Results	Sample_ Name
C1	-2.362	0.3	0.298	NEG	NEG Control
C2	-2.362	0.3	0.347	POS	POS control
S3	-2.362	0.3	0.337	POS	20
S4	-2.362	0.3	0.333	POS	20
S5	-2.362	0.3	0.336	POS	20
S6	-2.362	0.3	0.338	POS	20
S7	-2.362	0.3	0.331	POS	20
S8	-2.362	0.3	0.334	POS	20
S9	-2.362	0.3	0.333	POS	20
S10	-2.362	0.3	0.335	POS	20
S11	-2.362	0.3	0.334	POS	20
S12	-2.362	0.3	0.337	POS	20
S13	-2.362	0.3	0.338	POS	20
S14	-2.362	0.3	0.333	POS	20
S15	-2.362	0.3	0.338	POS	20
S16	-2.362	0.3	0.336	POS	20
S17	-2.362	0.3	0.335	POS	20
S18	-2.362	0.3	0.336	POS	20
S19	-2.362	0.3	0.331	POS	20
S20	-2.362	0.3	0.332	POS	20
S21	-2.362	0.3	0.331	POS	20
S22	-2.362	0.3	0.335	POS	20
S23	-2.362	0.3	0.3	NEG	0
S24	-2.362	0.3	0.3	NEG	0
S25	-2.362	0.3	0.299	NEG	0
S26	-2.362	0.3	0.3	NEG	0
S27	-2.362	0.3	0.3	NEG	0
S28	-2.362	0.3	0.299	NEG	0
S29	-2.362	0.3	0.3	NEG	0
S30	-2.362	0.3	0.298	NEG	0
S31	-2.362	0.3	0.3	NEG	0
S32	-2.362	0.3	0.3	NEG	0
S33	-2.362	0.3	0.3	NEG	0
S34	-2.362	0.3	0.298	NEG	0
S35	-2.362	0.3	0.3	NEG	0
S36	-2.362	0.3	0.3	NEG	0
S37	-2.362	0.3	0.3	NEG	0
S38	-2.362	0.3	0.3	NEG	0
S39	-2.362	0.3	0.3	NEG	0
S40	-2.362	0.3	0.297	NEG	0
S41	-2.362	0.3	0.3	NEG	0
S42	-2.362	0.3	0.299	NEG	0

Operator: Arsh
ReaderID: R6

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Table 2: Test Results for PE Nucleic Acid Detection Kit and Kaya17 nCoVega Test.

Sample	N Gene	IC	ORF1ab	PE RESULT	Kaya17 (RFU)	Kaya17 RESULT	Operator
NP-1	UND	36.77	UND	NOT DETECTED	0.275	NEG	
NP-2	30.05	33.47	30.07	DETECTED	0.392	POS	
NP-3	UND	33.84	UND	NOT DETECTED	0.266	NEG	
NP-4	UND	32.56	UND	NOT DETECTED	0.268	NEG	
NP-5	UND	33.47	UND	NOT DETECTED	0.289	NEG	
NP-6	UND	34.90	UND	NOT DETECTED	0.292	NEG	
NP-7	UND	33.59	UND	NOT DETECTED	0.284	NEG	
NP-8	22.87	33.53	22.67	DETECTED	0.64	POS	
NP-9	UND	33.64	UND	NOT DETECTED	0.281	NEG	
NP-10	30.59	35.99	31.96	DETECTED	0.293	NEG	
NP-11	31.49	33.79	UND	DETECTED	0.385	POS	
NP-12	33.10	33.56	35.57	DETECTED	0.382	POS	
NP-13	24.81	33.01	25.53	DETECTED	0.562	POS	
NP-14	29.64	34.02	29.71	DETECTED	0.396	POS	
NP-15	19.83	32.21	20.71	DETECTED	0.767	POS	
NP-16	UND	31.14	UND	NOT DETECTED	0.279	NEG	
NP-17	UND	33.67	UND	NOT DETECTED	0.284	NEG	
NP-18	UND	33.15	UND	NOT DETECTED	0.278	NEG	1
NP-19	27.92	32.49	23.68	DETECTED	0.446	POS	
NP-20	UND	33.75	UND	NOT DETECTED	0.288	NEG	
NP-21	32.94	33.16	34.98	DETECTED	0.383	POS	
NP-22	UND	32.56	UND	NOT DETECTED	0.295	NEG	
NP-23	17.86	33.58	17.55	DETECTED	0.845	POS	
NP-24	UND	33.63	UND	NOT DETECTED	0.287	NEG	
NP-25	UND	32.89	UND	NOT DETECTED	0.293	NEG	
NP-26	UND	34.45	UND	NOT DETECTED	0.276	NEG	
NP-27	27.42	33.36	26.99	DETECTED	0.448	POS	
NP-28	UND	33.31	UND	NOT DETECTED	0.296	NEG	
NP-29	32.99	32.23	34.33	DETECTED	0.388	POS	
NP-30	19.55	33.49	19.75	DETECTED	0.763	POS	
NP-31	UND	31.48	UND	NOT DETECTED	0.323	POS	
NP-32	UND	32.89	UND	NOT DETECTED	0.293	NEG	
NP-33	UND	33.02	UND	NOT DETECTED	0.278	NEG	
NP-34	28.52	33.94	28.70	DETECTED	0.38	POS	
NP-35	33.95	33.58	34.86	DETECTED	0.376	POS	

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NP-36	UND	34.46	UND	NOT DETECTED	0.278	NEG	
NP-37	UND	33.92	UND	NOT DETECTED	0.294	NEG	
NP-38	UND	32.53	UND	NOT DETECTED	0.288	NEG	
NP-39	28.82	35.67	28.30	DETECTED	0.401	POS	
NP-40	UND	36.41	UND	NOT DETECTED	0.287	NEG	
NP-41	UND	33.23	UND	NOT DETECTED	0.298	NEG	
NP-42	UND	32.34	UND	NOT DETECTED	0.272	NEG	
NP-43	UND	33.63	UND	NOT DETECTED	0.284	NEG	
NP-44	UND	32.22	UND	NOT DETECTED	0.281	NEG	
NP-45	UND	34.12	UND	NOT DETECTED	0.293	NEG	
NP-46	UND	33.41	UND	NOT DETECTED	0.285	NEG	
NP-47	UND	33.30	UND	NOT DETECTED	0.296	NEG	
NP-48	UND	33.20	UND	NOT DETECTED	0.294	NEG	
NP-49	UND	33.46	UND	NOT DETECTED	0.294	NEG	
NP-50	UND	34.85	UND	NOT DETECTED	0.281	NEG	
NP-51	UND	33.56	UND	NOT DETECTED	0.283	NEG	2
NP-52	UND	32.66	UND	NOT DETECTED	0.289	NEG	
NP-53	UND	33.96	UND	NOT DETECTED	0.288	NEG	
NP-54	34.32	32.80	34.16	DETECTED	0.375	POS	
NP-55	UND	33.93	UND	NOT DETECTED	0.279	NEG	
NP-56	35.10	32.53	35.91	DETECTED	0.379	POS	
NP-57	18.76	33.67	19.82	DETECTED	0.817	POS	
NP-58	34.40	32.93	33.86	DETECTED	0.387	POS	
NP-59	UND	32.79	UND	NOT DETECTED	0.286	NEG	
NP-60	UND	34.54	UND	NOT DETECTED	0.294	NEG	
NP-61	UND	33.64	UND	NOT DETECTED	0.287	NEG	
NP-62	UND	35.73	UND	NOT DETECTED	0.295	NEG	
NP-63	UND	33.16	UND	NOT DETECTED	0.291	NEG	
NP-64	UND	35.99	UND	NOT DETECTED	0.296	NEG	
NP-65	UND	32.35	UND	NOT DETECTED	0.279	NEG	

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NP-66	UND	33.90	UND	NOT DETECTED	0.289	NEG	
NP-67	UND	34.65	UND	NOT DETECTED	0.293	NEG	
NP-68	23.55	33.55	24.67	DETECTED	0.675	POS	
NP-69	34.74	32.82	35.92	DETECTED	0.377	POS	
NP-70	25.66	33.78	UND	DETECTED	0.555	POS	
NP-71	26.11	33.00	28.00	DETECTED	0.479	POS	
NP-72	UND	34.75	UND	NOT DETECTED	0.288	NEG	
NP-73	UND	35.03	UND	NOT DETECTED	0.284	NEG	
NP-74	35.16	33.87	36.84	DETECTED	0.368	POS	
NP-75	35.30	33.85	33.48	DETECTED	0.379	POS	
NP-76	UND	35.00	UND	NOT DETECTED	0.288	NEG	
NP-77	UND	34.78	UND	NOT DETECTED	0.292	NEG	
NP-78	UND	33.98	UND	NOT DETECTED	0.296	NEG	
NP-79	UND	35.67	UND	NOT DETECTED	0.289	NEG	
NP-80	UND	34.79	UND	NOT DETECTED	0.293	NEG	
NP-81	39.23	33.18	UND	DETECTED	0.329	POS	
NP-82	35.63	33.12	34.82	DETECTED	0.366	POS	3
NP-83	UND	34.63	UND	NOT DETECTED	0.287	NEG	
NP-84	UND	34.56	UND	NOT DETECTED	0.276	NEG	
NP-85	UND	33.78	UND	NOT DETECTED	0.293	NEG	
NP-86	18.87	34.01	19.05	DETECTED	0.842	POS	
NP-87	25.12	33.43	24.93	DETECTED	0.528	POS	
NP-88	26.76	33.72	26.40	DETECTED	0.492	POS	
NP-89	UND	32.91	UND	NOT DETECTED	0.294	NEG	
NP-90	UND	33.54	UND	NOT DETECTED	0.296	NEG	
NP-91	33.74	31.96	34.55	DETECTED	0.39	POS	
NP-92	28.52	33.60	29.88	DETECTED	0.421	POS	
NP-93	35.47	33.66	35.45	DETECTED	0.376	POS	
NP-94	22.45	33.41	21.77	DETECTED	0.674	POS	
NP-95	UND	33.03	UND	NOT DETECTED	0.286	NEG	
NP-96	UND	32.91	UND	NOT DETECTED	0.288	NEG	
NP-97	UND	35.30	UND	NOT DETECTED	0.294	NEG	
NP-98	UND	33.84	UND	NOT DETECTED	0.295	NEG	

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NP-99	UND	34.77	UND	NOT DETECTED	0.293	NEG	
NP-100	34.33	32.07	34.50	DETECTED	0.374	POS	
NP-101	UND	32.10	UND	NOT DETECTED	0.295	NEG	
NP-102	UND	32.21	UND	NOT DETECTED	0.296	NEG	
NP-103	UND	32.42	UND	NOT DETECTED	0.287	NEG	
NP-104	34.46	32.29	34.85	DETECTED	0.371	POS	
NP-105	37.67	32.55	38.11	DETECTED	0.368	POS	
NP-106	UND	31.77	UND	NOT DETECTED	0.288	NEG	
NP-107	UND	34.54	UND	NOT DETECTED	0.279	NEG	
NP-108	UND	33.32	UND	NOT DETECTED	0.293	NEG	
NP-109	UND	33.65	UND	NOT DETECTED	0.294	NEG	
NP-110	35.98	32.73	36.01	DETECTED	0.366	POS	
NP-111	34.66	33.21	34.97	DETECTED	0.37	POS	
NP-112	26.43	34.88	27.98	DETECTED	0.487	POS	
NP-113	30.12	31.44	UND	DETECTED	0.396	POS	
NP-114	UND	32.28	UND	NOT DETECTED	0.285	NEG	
NP-115	UND	32.69	UND	NOT DETECTED	0.296	NEG	
NP-116	UND	34.55	UND	NOT DETECTED	0.288	NEG	
NP-117	UND	33.99	UND	NOT DETECTED	0.329	POS	4
NP-118	UND	33.24	UND	NOT DETECTED	0.295	NEG	
NP-119	UND	33.40	UND	NOT DETECTED	0.293	NEG	
NP-120	UND	32.59	UND	NOT DETECTED	0.289	NEG	
NP-121	31.54	32.73	30.98	DETECTED	0.387	POS	
NP-122	33.39	33.11	33.28	DETECTED	0.379	POS	
NP-123	UND	36.46	UND	NOT DETECTED	0.297	NEG	
NP-124	22.85	33.26	22.37	DETECTED	0.646	POS	
NP-125	UND	33.55	UND	NOT DETECTED	0.288	NEG	
NP-126	UND	31.67	UND	NOT DETECTED	0.285	NEG	
NP-127	UND	34.21	UND	NOT DETECTED	0.295	NEG	
NP-128	UND	33.44	UND	NOT DETECTED	0.296	NEG	
NP-129	UND	32.45	UND	NOT DETECTED	0.292	NEG	
NP-130	14.73	32.65	15.10	DETECTED	1.272	POS	
NP-131	21.58	33.32	21.99	DETECTED	0.683	POS	
NP-132	33.97	33.48	35.56	DETECTED	0.384	POS	
NP-133	35.25	33.77	36.95	DETECTED	0.368	POS	
NP-134	32.89	34.25	34.22	DETECTED	0.381	POS	
NP-135	35.33	33.92	35.02	DETECTED	0.374	POS	

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NP-136	37.74	34.67	38.08	DETECTED	0.361	POS	
NP-137	15.76	32.69	15.59	DETECTED	0.921	POS	
NP-138	UND	32.29	UND	NOT DETECTED	0.285	NEG	
NP-139	UND	31.90	UND	NOT DETECTED	0.296	NEG	
NP-140	UND	32.65	UND	NOT DETECTED	0.293	NEG	
NP-141	UND	32.98	UND	NOT DETECTED	0.297	NEG	
NP-142	35.17	32.28	34.66	DETECTED	0.327	POS	
NP-143	UND	33.58	UND	NOT DETECTED	0.295	NEG	
NP-144	33.75	33.88	33.21	DETECTED	0.384	POS	
NP-145	33.76	32.55	34.86	DETECTED	0.388	pos	
NP-146	29.58	33.75	29.57	DETECTED	0.397	POS	
NP-147	21.65	33.45	21.50	DETECTED	0.683	POS	
NP-148	39.00	32.71	37.06	DETECTED	0.328	POS	
NP-149	UND	31.90	UND	NOT DETECTED	0.296	NEG	
NP-150	34.79	32.56	36.85	DETECTED	0.374	POS	
NP-151	35.02	32.32	34.62	DETECTED	0.377	POS	
NP-152	UND	34.56	UND	NOT DETECTED	0.293	NEG	
NP-153	UND	33.67	UND	NOT DETECTED	0.293	NEG	
NP-154	UND	33.84	UND	NOT DETECTED	0.287	NEG	
NP-155	35.22	32.18	36.37	DETECTED	0.374	POS	
NP-156	34.82	33.00	34.67	DETECTED	0.375	POS	
NP-157	34.48	32.91	34.44	DETECTED	0.372	POS	
NP-158	UND	33.10	UND	NOT DETECTED	0.296	NEG	
NP-159	UND	33.87	UND	NOT DETECTED	0.293	NEG	
NP-160	UND	33.04	UND	NOT DETECTED	0.295	NEG	5
NP-161	UND	32.29	UND	NOT DETECTED	0.294	NEG	
NP-162	UND	33.46	UND	NOT DETECTED	0.295	NEG	
NP-163	UND	33.85	UND	NOT DETECTED	0.297	NEG	
NP-164	UND	34.06	UND	NOT DETECTED	0.294	NEG	
NP-165	UND	31.54	UND	NOT DETECTED	0.295	NEG	
NP-166	36.03	33.01	34.70	DETECTED	0.365	POS	
NP-167	UND	30.97	38.06	DETECTED	0.328	POS	
NP-168	UND	33.15	38.87	DETECTED	0.325	POS	
NP-169	35.31	32.80	35.16	DETECTED	0.376	POS	
NP-170	37.82	33.47	38.92	DETECTED	0.365	POS	
NP-171	30.79	34.47	29.69	DETECTED	0.392	POS	
NP-172	31.62	33.25	32.08	DETECTED	0.383	POS	
NP-173	UND	36.19	UND	NOT DETECTED	0.297	NEG	
NP-174	26.75	34.21	26.73	DETECTED	0.482	POS	
NP-175	27.52	33.68	27.59	DETECTED	0.447	POS	
NP-176	29.30	34.07	29.27	DETECTED	0.398	POS	
NP-177	UND	33.13	UND	NOT DETECTED	0.293	NEG	
NP-178	33.56	33.41	36.53	DETECTED	0.38	POS	
NP-179	35.30	32.72	35.66	DETECTED	0.372	POS	
NP-180	34.86	33.66	36.34	DETECTED	0.369	POS	
NP-181	35.80	33.63	37.02	DETECTED	0.364	POS	
NP-182	UND	34.56	UND	NOT DETECTED	0.292	NEG	
NP-183	UND	33.55	UND	NOT DETECTED	0.297	NEG	

Citation: Camille Troup, Debnath Mukhopadhyay, Tania Chakrabarty, Anup Madan, Sri Satyanarayana, Vel Murugan and Su Dwarakanath. Development of an Accurate and Rapid Antigen Assay for COVID-19 Diagnostics Using Saliva. Archives of Clinical and Biomedical Research. 7 (2023): 133-146.

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matrix obtained from healthy donors and confirmed negative for SARS-CoV-2. At each dilution, 500µL samples were added to Extraction buffer and tested using the nCoVega test.

Clinical Evaluation

A prospective, single center, comparative study for validation of a rapid point-of-care test for diagnosis of SARS-COV-2 infection was conducted to evaluate the clinical use of the Kaya17 nCoVega COVID-19 Antigen test for use with saliva specimens collected neat. The Kaya17 nCoVega COVID-19 Antigen test was clinically validated by Infinity BiologiX (IBX) using 183 paired saliva and swab samples (79 positives and 104 negatives) collected by St. Rose Hospital, Hayward, CA as per KAYA-PROTOPOCT-007 Prospective Clinical Study Protocol. The Western IRB approval number for the clinical trial was IRB protocol #20204097. Informed consents were collected from each subject along with basic medical history. The samples utilized in the study were analyzed using an RT PCR assay (Control), PerkinElmer New Coronavirus Nucleic Acid Detection Kit. The primary objective of the study was to demonstrate efficacy of the Kaya17 nCoVega COVID-19 Antigen test in a non-laboratory setting by verifying the sensitivity and specificity of the Kaya17 nCoVega COVID-19 Antigen test in detecting SARS-COV-2 in positive saliva samples (yields a "positive" result) and not detecting SARS-COV-2 in negative saliva samples (yields a "negative" result) when performed by non-laboratory personnel. The secondary objective in the same protocol was to demonstrate that non-laboratory healthcare providers can perform the Kaya17 nCoVega COVID-19 Antigen test accurately in the intended use environment to Support Point of Care (POC) Use. (Point of Care study)

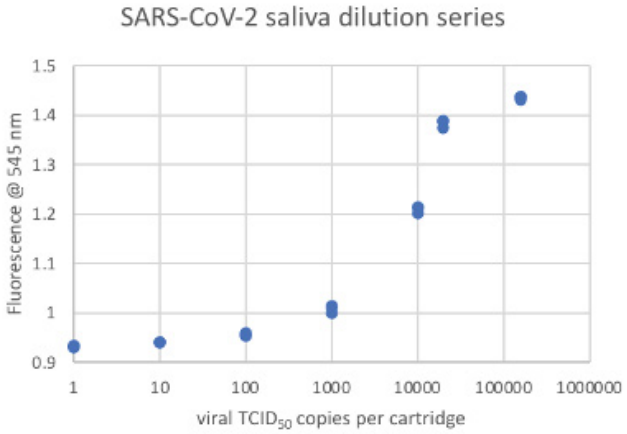

Figure 2 (a): LoD determination and dynamic range based on data from Table 1 (see supplementary section).

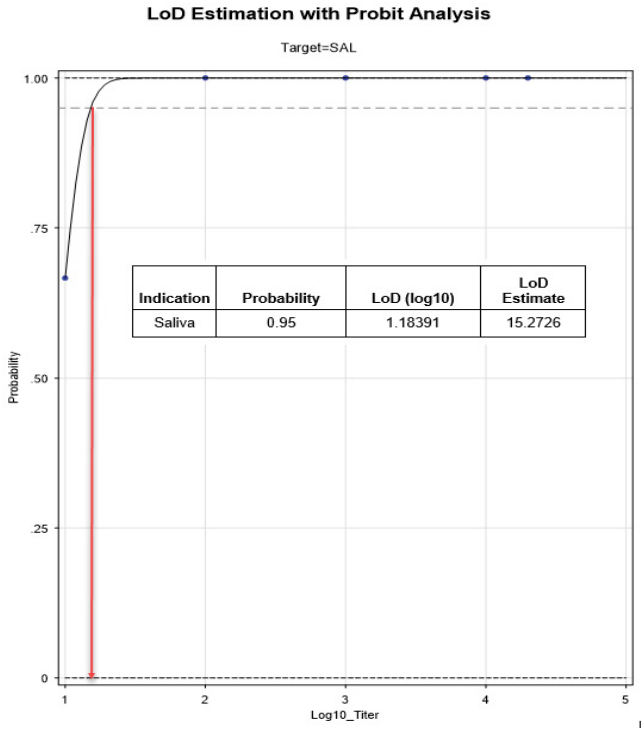

Figure 2 (b): Probit Analysis to determine LoD using data from Table 1.

The data generated from the clinical study were statistically analyzed as per the following techniques

- Positive percent agreement (PPA),
- Negative percent agreement (NPA),
- Overall percent agreement (OPA),
- Associated 95% Wilson score confidence intervals
- Concordance analysis of the Kaya17 nCoVega Antigen test RFU vs Ct values of PCR

Subject status (negative/positive) was determined using a receiver operating characteristic (ROC) curve cutoff analysis. The evaluation metrics included positive percent agreement (PPA), negative percent agreement (NPA), overall percent agreement (OPA), Youden index, distance to (0,1), and the absolute value of the sensitivity minus the specificity. Before testing any of the samples, sample cartridges with positive and negative controls were inserted on the Vega 200 instrument that recorded fluorescence from these samples in RFU. This allowed the Vega analysis software to decide the cut-off value to use when deciding on positive and negative COVID-19 calls for sample testing. Details of the result interpretation are cited below in their respective sections. Raw data for the plots are also included in the supplementary information section. All test controls were examined prior to interpretation of results. If the controls were not valid, the samples were not analyzed using the kit/instrument. Additional kits or instruments are required to be validated prior to running samples. The test result was determined by cutoff values based on fluorescence measurements on the Vega-200 instrument (excitation at 395 nm and emission at 545 nm). A "POSITIVE" result is called by the Vega software if the fluorescence measurement of the sample is above baseline fluorescence that has been

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determined by running the negative control multiple times. If the fluorescence value is at or below baseline, a "NEGATIVE" result is called by the Vega software. The results are stored in a CSV file on the Vega Reader computer. Further details are provided in the supplementary section.

Results

For laboratory validation of the nCoVega COVID19 Antigen test, a number of different studies were conducted such as analytical performance testing, LOD, cross-reactivity, inclusion and exclusion panel testing and hook effect testing. The details of each of those tests served to establish the sensitivity and specificity of the assay and the results are presented below.

Microorganism	Source	Concentration	Cross-Reactivity (Yes/No)
Human coronavirus 229E	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 negative)
Human coronavirus OC43	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
Human coronavirus NL63	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
MERS coronavirus	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
Adenovirus	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
Human Metapneumovirus (hMPV)	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
Parainfluenza virus Type 1	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
Parainfluenza virus Type 2	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
Parainfluenza virus Type 3	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
Parainfluenza virus Type 4a	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
Influenza A H3N2 (Wisconsin/67/05)	BEI Resources	5.4 x 10^4 $TCID_{50}$/mL	No (3/3 Negative)
Influenza A H1N1	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
Influenza B (Florida/02/06)	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
Enterovirus Type 68	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
Respiratory syncytial Virus Type B	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
Rhinovirus	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
SARS-CoV	BEI Resources	1x10^5 PFU/mL	No (3/3 Negative)
Herpes Simplex Virus	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 Negative)
Epstein Barr Virus	Zeptometrix	1.03x10^3cp/mL	No (3/3 Negative)
Haemophilus influenzae	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Streptococcus pneumoniae	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Streptococcus pyogenes	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Candida albicans	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Bordetella pertussis	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Chlamydophila pneumoniae	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Legionella pneumophila	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Mycobacterium tuberculosis	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Pneumocystis jirovecii	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Pseudomonas Aeruginosa	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Staphylococcus Epidermidis	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Streptococcus Salivarius	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Mycoplasma pneumoniae	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Pooled human Kaya17 nasal wash	Kaya17	14% v/v	No (3/3 Negative)
Elkenella corrodens	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Streptococcus mutans	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Staphlocccus aureus	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Neisseria Meningitidis	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Nocardia asteriods	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Moraxella catarrhalis	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)
Streptococcus mitis	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 Negative)

Table 3: Results of Cross-Reactivity Study

Microorganism	Source	Concentration	Interference (Yes/No)
Human coronavirus 229E	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
Human coronavirus OC43	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
Human coronavirus NL63	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
MERS coronavirus	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
Adenovirus	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
Human Metapneumovirus (hMPV)	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
Parainfluenza virus Type 1	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
Parainfluenza virus Type 2	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
Parainfluenza virus Type 3	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
Parainfluenza virus Type 4a	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
Influenza A H3N2 (Wisconsin/67/05)	BEI Resources	5.4 x 10^4 $TCID_{50}$/mL	No (3/3 positive)
Influenza A H1N1	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
Influenza B (Florida/02/06)	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
Enterovirus Type 68	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
Respiratory syncytial Virus Type B	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
Rhinovirus	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
SARS-CoV	BEI Resources	1x10^5 PFU/mL	No (3/3 positive)
Herpes Simplex Virus	Zeptometrix	1 x 10^5 $TCID_{50}$/mL	No (3/3 positive)
Epstein Barr Virus	Zeptometrix	1.03x10^3cp/mL	No (3/3 positive)
Haemophilus influenzae	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 positive)
Streptococcus pneumoniae	Zeptometrix	1 x 10^6 CFU/mL	No (3/3 positive)

Table 4: Results of Microbial Interference studies

Analytical Performance

Limit of Detection (LoD) Determination: LoD studies were performed to determine the lowest detectable concentration of SARS-CoV-2 at which approximately 95% of all (true positive) replicates test positive.

From the ten-fold dilution series results in Table 1, the LoD can be determined as at least 0.95 (100 TCID50/mL) since all samples with concentrations from 100000 TCID50/mL down to 100 TCID50/mL were positive based on the 0.94 cutoff. The ability to detect SARS-CoV-2 with Kaya17 nCoVega test at 20 $TCID_{50}$ per ml was further confirmed by testing 20 replicates (Table 1 in supplementary section). Limit of Blank (LoB) experiments were performed by running 20 samples at 0 TCID50 per ml LOB (Table 1 in supplementary section).

Assay Cutoff Determination: Determining the assay cutoff was a key part of the data analysis. The assay cutoff determination for positives and negatives is determined using the Currie's method11 and CLSI EP17 guidance12 for our qualitative purposes as follows.

From the guidance, the LoB' is defined as:

where M refers to the mean of "blank" samples and Cp is the multiplier and SDB is the Standard Deviation of the Blank Samples. As the data are very precise, in this case using a two-sided 5% significance level, Cp is defined as

where B is the total number of blank replicates and K is the number of blank samples.

Therefore, from the data in the spreadsheet (see supplementary section), the LOB' is calculated as:

Citation: Camille Troup, Debnath Mukhopadhyay, Tania Chakrabarty, Anup Madan, Sri Satyanarayana, Vel Murugan and Su Dwarakanath. Development of an Accurate and Rapid Antigen Assay for COVID-19 Diagnostics Using Saliva. Archives of Clinical and Biomedical Research. 7 (2023): 133-146.

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and SDL = 0.00266 is the standard deviation of the positive low concentration samples with appropriate provision for degrees of freedom depending on the number of samples used to obtain the total replicates. Therefore, if the LoB' is taken as the cutoff (11), then we can establish a conservative equivocal zone between the LoB' and the LoD' as between 0.936 and 0.942 mean RR with the actual cutoff as 0.939 (as determined by ROC curve methodology in clinical evaluation (12)). Therefore, if a patient were to test in this equivocal zone, their result should be assigned as INDETERMINATE and a retest is performed.

Cross-Reactivity (Analytical Specificity): We performed cross-reactivity studies for Kaya17 nCoVega test using a panel of related pathogens, normal or pathogenic flora that are reasonably found in the clinical specimen. Also, the high prevalence disease agents likely to be encountered in the clinical specimen were tested for specificity of the test and the results showed no potential cross-reactivity with the Kaya17 nCoVega test with these agents, including various microorganisms and pathogens.

Microbial Interference Studies: Microbial Interference for Kaya17 nCoVega test was evaluated by using a panel of related pathogens, normal or pathogenic flora that are reasonably found in the clinical specimen. Also, high prevalence disease agents were tested for specificity to demonstrate that false negatives do not occur when SARS-CoV-2 is present in a specimen with other microorganisms.

Endogenous Interference Substances Studies: The following study was conducted to investigate whether potentially interfering substances, which may be found in the mouth and throat of symptomatic subjects (including over-the-counter medications), cross-react or interfere with the detection of SARS-CoV-2 using the Kaya17 nCoVega test. There was no interference with any of the tested materials in the study as described in Table 5.

High-dose Hook Effect: The Weibull statistical model was used for the data analysis (x axis=log10 $TCID_{50}$ copies; y axis =Raw Average). The analysis showed that at the top end of the assay (1.6 x 10^5 $TCID_{50}$ copies) there is a plateau. This provides evidence that a Hook effect does not exist for this assay up to 1.6 x 10^5 $TCID_{50}$ copies. The model fits, as measured by the correlation and coefficient of determination, were each about 0.99 (closer to 1.00 is desired). Specific model information is shown in the figure 3.

Clinical Evaluation

A total of n=183 samples were collected during the clinical evaluation. A total of n=183 test samples utilizing the subject device resulted in the same result (i.e., positive or negative) in comparison to the control, except two (n=2) test sample rendered a positive result when using the subject device and a negative result when using the control. One (n=1) test samples rendered a negative result when using the subject device and a positive result when using the control. After conducting statistical analysis, a 98.4% degree of accuracy is established based on the data set (Table 6).

Accuracy in Overall Patient Population: The test results include 79 True Positives (RT-PCR Positives) and 104 True negatives (RT-PCR Negatives). When we analyzed the performance of the Kaya17 assay in overall population, the PPA value is calculated to be 98.7% and at 95% Confidence Interval, values are found to be at (93.2% Lower bound, 99.8% Upper bound).

Accuracy in Symptomatic Patient Population: In the Symptomatic population (Table 7), there are 73 RT-PCR positives (True Positives) and 45 RT-PCR negatives (True Negatives). When we analyzed the performance of the Kaya17 assay in symptomatic population, the PPA value is calculated to be 98.6% and at *95% Confidence Interval*, values are found to be at (92.60% Lower bound, 99.8% Upper bound). It meets the FDA's requirement for symptomatic population that positive percent agreement (PPA) of at least 80% with 70% at the lower bound of the two-sided 95% confidence interval, in symptomatic patients suspected of COVID-19 infection by their healthcare providers.

Accuracy in Asymptomatic Patient Population: In the Asymptomatic population (Table 8), there are 6 RT-PCR positives (True Positives) and 59 RT-PCR negatives

Interfering Substance	Concentration	Interference (Yes/No)
Clarispray	50mcg	No (3/3 Negative, 3/3 Positive)
Blood (human)	5%	No (3/3 Negative, 3/3 Positive)
Mucin	5 mg/mL	No (3/3 Negative, 3/3 Positive)
Fluticasone Propionate Flovant HFA	110mcg	No (3/3 Negative, 3/3 Positive)
Ventolin HFA Albuterol	90mcg	No (3/3 Negative, 3/3 Positive)
Budesonide and Fromoterol Fumarate Dihydrate	80mcg/4.5mcg	No (3/3 Negative, 3/3 Positive)
Anefrin oxymetazoline HCl	0.05% v/v	No (3/3 Negative, 3/3 Positive)
Azelastine HCl	137mcg	No (3/3 Negative, 3/3 Positive)
Sore Throat Phenol Spray	15% v/v	No (3/3 Negative, 3/3 Positive)
Montelukast (Singular)	0.5mg	No (3/3 Negative, 3/3 Positive)
Laratadine (Claritin)	0.15 mg/dL	No (3/3 Negative, 3/3 Positive)
Human Genomic DNA	10 ug.mL	No (3/3 Negative, 3/3 Positive)
Nyquil (Acetaminophen, Doxylamine succinate, Dextromethorphan HBr)	2 mg/mL	No (3/3 Negative, 3/3 Positive)
Toothpaste (Colgate)	0.5% v/v	No (3/3 Negative, 3/3 Positive)

Table 5: Results of Interference Studies.

Citation: Camille Troup, Debnath Mukhopadhyay, Tania Chakrabarty, Anup Madan, Sri Satyanarayana, Vel Murugan and Su Dwarakanath. Development of an Accurate and Rapid Antigen Assay for COVID-19 Diagnostics Using Saliva. Archives of Clinical and Biomedical Research. 7 (2023): 133-146.

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(True Negatives). When we analyzed the performance of the Kaya17 assay in asymptomatic population, the PPA value is calculated to be 100% and at *95% Confidence Interval*, values are found to be at (61% Lower bound, 100% Upper bound).

Out of 183 clinical study samples (Table 9), 34 of them are *N gene* low positives and 31 of them are *ORF* low positives (calculated from the results of comparator test. i.e., PerkinElmer (PE) New Coronavirus Nucleic Acid Detection Kit RT-PCR). The PE RT-PCR LoD for *N gene* is 35.8 and the LoD for *ORF* is 37 as per the IFU of PE Nucleic Acid Detection Kit (provided as Annexure A to clinical protocol). Hence, we considered the Ct value range of 32.8 – 35.8 for *N gene* and the range of 34 – 37 for *ORF* to calculate the percentage of low positives from the overall clinical samples as recommended by FDA (Section J10 of *FDA Template for Developers of Antigen Tests under EUA dated 6th October 2021*) that low positives are defined as samples in which any gene target is within 3 cycle thresholds (Ct's) of the mean Ct count at the comparator test's LoD. The low positive values from the RT-PCR test results are highlighted in blue color in Table 7. The percentage of low positive samples are determined to be 18.58 % for *N gene* & 16.94% for *ORF* of the total sample size (183) which meets the FDA requirement of having approximately 10-20% of the clinical samples should be low positives in the clinical samples.

The operator specific accuracy is measured to support the POC Study to demonstrate that the Kaya17 nCoVega COVID-19 Antigen test can be performed by the untrained operator following the Quick Reference Guide without any training. Each untrained operator has achieved the 95% accuracy. Please refer to the Table 10.

Overview

Name	Weibull Model
Kind	Regression
Family	Sigmoidal Models
Equation	y = a - b*exp(-c*x^d)
# of Indep. Vars	1
Weighting	Default
Standard Error	0.022830668235798594
Correlation Coeff. (r)	0.994782
Coeff. of Determination (r^2)	0.9895911573703048
DOF	17
AICC	-155.771049

Parameters

	Value	Std Err	Range (95% confidence)
a	1.440984	0.013098	1.413350 to 1.468618
b	0.486479	0.015155	0.454504 to 0.518453
c	0.000000	0.000000	-0.000000 to 0.000000
d	11.748741	1.621902	8.326827 to 15.170655

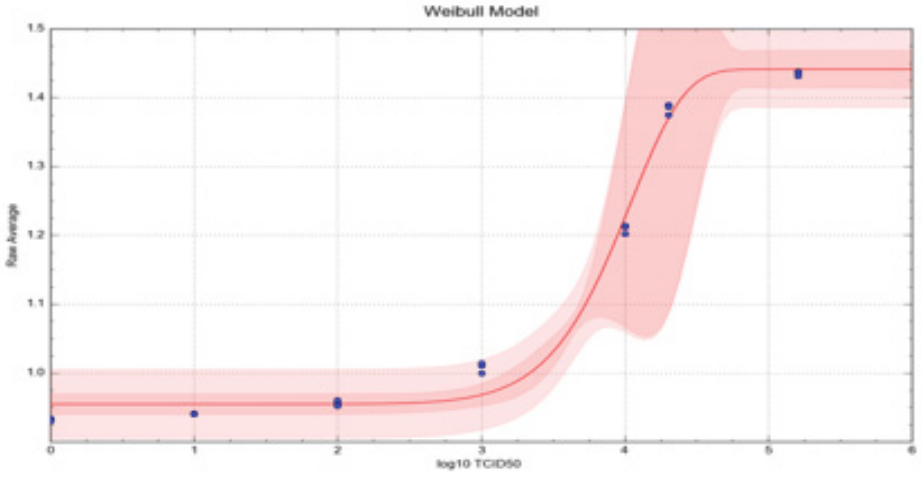

Figure 3: Weibull statistical analysis of Hook effect data.

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Table 6: Accuracy in Overall Patient Population.

		PCR Reference Result			Agreement		
Comparator Assay	**Test Result**	**Positive**	**Negative**	**Total**	**Measure [a]**	**% (n/N)**	**95% CI [b]**
Kaya17 (Overall)	Positive	78	2	80	PPA	98.7 (78/79)	(93.2, 99.8)
	Negative	1	102	103	NPA	98.1 (102/104)	(93.3, 99.5)
	Total	79	104	183	OPA	98.4 (180/183)	(95.3, 99.4)

[a] PPA=Positive Percent Agreement, NPA=Negative Percent Agreement, OPA=Overall Percent Agreement.
[b] Wilson Score Confidence Interval.

Table 7: Accuracy in Symptomatic Patient Population.

		PCR Reference Result			Agreement		
Comparator Assay	**Test Result**	**Positive**	**Negative**	**Total**	**Measure [a]**	**% (n/N)**	**95% CI [b]**
Kaya17 (Symptomatic)	Positive	72	1	73	PPA	98.6 (72/73)	(92.6, 99.8)
	Negative	1	44	45	NPA	97.8 (44/45)	(88.4, 99.6)
	Total	73	45	118	OPA	98.3 (116/118)	(94.0, 99.5)

[a] PPA=Positive Percent Agreement, NPA=Negative Percent Agreement, OPA=Overall Percent Agreement.
[b] Wilson Score Confidence Interval.

Table 8: Accuracy in Asymptomatic Patient Population.

		PCR Reference Result			Agreement		
Comparator Assay	**Test Result**	**Positive**	**Negative**	**Total**	**Measure [a]**	**% (n/N)**	**95% CI [b]**
Kaya17 (Asymptomatic)	Positive	6	1	7	PPA	100 (6/6)	(61.0, 100.0)
	Negative	0	58	58	NPA	98.3 (58/59)	(91.0, 99.7)
	Total	6	59	65	OPA	98.5 (64/65)	(91.8, 99.7)

[a] PPA=Positive Percent Agreement, NPA=Negative Percent Agreement, OPA=Overall Percent Agreement.
[b] Wilson Score Confidence Interval.

Table 9: Low Positives in RT-PCR Results.

Total	**N Gene Low Positives (Ct Value range: 32.8 - 35.8)**	**ORF Low Positives (Ct Value range: 34 - 37)**
	34	31
Percentage w.r.t Overall Sample size (183)	18.58%	16.94%

Table 10: It is also observed that there is no reader instrument variability while interpreting the results when the samples are tested. All the RT-PCR comparative results correlate with the Kaya17 results when 5 different reader instruments were used for testing by 5 different untrained operators.

S.No	**Operator**	**Total No. Samples Tested**	**Correctly measured w.r.t Control**	**Accuracy (%)**
1	Operator 1	41	39	95.1
2	Operator 2	36	36	100
3	Operator 3	39	39	100
4	Operator 4	43	42	97.7
5	Operator 5	54	54	100

Figure 4 shows the inverse linear relationship between the observed Kaya17 RFUs versus the mean values of the N and ORF1ab Gene cycle thresholds (n=183, Pearson Correlation= -0.92). The figure shows that all of the negative samples by PCR were also Negative in Kaya17 nCoVega test, except for two samples (Sample Number 31and 117), all positive samples by PCR were also positive by Kaya17 nCoVega test, except for one sample (Sample Number 10).

Discussion

The Kaya17 Vega-200 system and nCoVega antigen test is a simple-to-use, rapid assay that is also highly sensitive and specific. The system can process 30-50 samples in one hour with minimal hands-on time. It can also detect very low levels of SARS-CoV-2 as seen in the high correlation with RT-PCR across all ranges of viral loads, all the way down to

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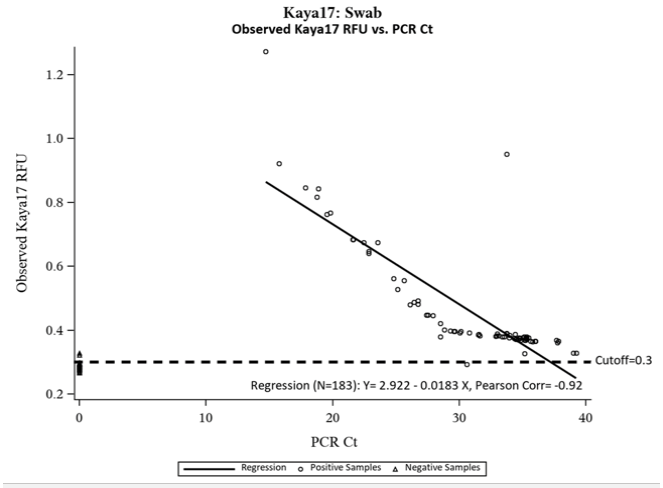

Figure 4: Inverse Relationship between Kaya17 RFUs vs Ct values from RT-PCR.

Ct counts of 38. The reader instrument is light, portable and runs off USB-power from its connected laptop, so it does not need external power source. Our end-to-end solution can be deployed in mobile units, at schools, offices and events and also in remote areas, with its small footprint. Easily scalable manufacturing processes llow this test to meet any surge in demand for COVID-19 testing as we open our schools, businesses and global travel and new variants emerge. In the future, this test system can be productized for self-testing purposes and for remote monitoring via a mobile app. The nCoVega test can address the unmet need for rapid, accurate and inexpensive COVID-19 testing and it is suitable for broader public dissemination.

Author Contributions

SD and VM conceived the study and cross-platform validation experiments at ASU. SS led the design and development of the Vega-200 system. SD, CBT, TC, AM, DM developed and optimized the nCoVega SARS-CoV-2 assay. DM and SD ran the analytical study. SD, CBT ran the clinical validation study at ASU. JC, JM and CBT analyzed the data. TC, SM and SD drafted the manuscript. CBT revised the manuscript. All authors read and approved the final manuscript.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Western IRB (IRB protocol #20204097; Kaya17 protocol #KAYA-PROTOPOCT-007 Rev002) on 01/06/2021.

Supplementary Information

Download the supplementary information from https://www.fortunejournals.com/supply/ACBR_7871.pdf

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