TROUBLESHOOTING

Problem	Possible Causes	Solutions
Specimen mixture is flowing too slowly through test device	Test device exposed to high humidity environment for too long	Follow test instructions for use. Perform the test as soon as possible after opening the cassette aluminum pouch.
	Temperuature of test environment is too low. The product performance has been validated between 15°C and 40°C.	Perform test at a temperature of between 15°C and 40°C.
Control line failes to appear	Product may have expired or the cassette strip is broken.	Use an unexpired and non-broken product.
Control line color development is weak	Product and/or test samples are at too cool a temperature	Prewarm both product and test sample to room temperature (about 20°C). Prewarm cassette before opening it and use it as soon as possible after opening the packing aluminum pouch.
	Temperuature of test environment is too low for good performance.	Perform test at a temperature of between 15°C and 40°C.

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3 Mulligan, M. J. *et al.* Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature* 586, 589-593, doi:10.1038/s41586-020-2639-4 (2020).
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5 Bergwerk, M. *et al.* Covid-19 Breakthrough Infections in Vaccinated Health Care Workers. *New England Journal of Medicine*, doi:10.1056/NEJMoa2109072 (2021).
6 Chia, W. N. *et al.* Dynamics of SARS-CoV-2 neutralising antibody responses and duration of immunity: a longitudinal study. *Lancet Microbe* 2, e240-e249, doi:10.1016/S2666-5247(21)00025-2 (2021).
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CoNAb SARS-CoV-2 NAb Lateral Flow Test Kit

REF	Catalog Number	LOT	Batch Code
	Consult Instructions for Use	4°C 30°C	Temperature Limitations
	Use by: YYYY-MM-DD	Σ	Contains sufficent for < n > tests
	Single Use Only		Manufacturer

NOVODIAX

CoNAb™ SARS-CoV-2 Neutralizing Antibody Lateral Flow Test

PRODUCT DESCRIPTION

CoNAb™ SARS-CoV-2 Neutralizing Antibody Lateral Flow Test (CoNAb) is to detect an individual's neutralizing antibodies (NAbs or NAb) to the COVID-19 virus.

BACKGROUND

NAbs are a subset of binding antibodies to SARS-CoV-2. They can be in any antibody classes, IgG, IgM, or IgA. A common feature they share is the capability of blocking the interaction between the spike protein of SARS-CoV-2 and its receptor, human angiotensin-converting enzyme 2 (ACE2), preventing the virus from invading into human host cells. It is known that NAbs often interact with a "supersite" in N-Terminal Domain (NTD)[1] and sites 1a and 1b in Receptor Binding Domain (RBD)[2]. Both NTD and RBD are located in S1 fragment, a subunit of spike protein. NAb level closely relates with vaccine effectiveness or protection rate and has been primary laboratory measure during any COVID-19 vaccine development[3,4]. Breakthrough cases (infection after full vaccination) mostly occurred among the people with lower levels of NAb[5].

Mean NAb level from convalescent patients ranges from 94[3] to 106[4] and the mean NAb peak level from vaccine recipients is 437 for BioNTech/Pfizer vaccne[3] and 654.3 for Moderna vaccine[4], both of which provide more than 90% protection from COVID-19 infection. However, the NAb titer after vaccination increases differently from person-to-person (e.g., 2.67-4.77 folds of that of convalescent patients, Table S6 of a cited reference[4]) and also declines from its peak level in an individual-dependent manner, either rapid waning, slow waning or persistent[6]. The knowledge about the relationship between NAb level and the protection against COVID-19 is still very limited. A predictive model of protective immunity against SARS-CoV-2 infection indicated that NAb level for 50% protection against detectable SARS-CoV-2 infection is estimated to be 20.2% of the mean convalescent level[7]. A convenient tool for measuring NAb can help in management of both public activities and personal life.

INTENDED USE *For Research Use Only*

CoNAb is a lateral flow test intended for qualitative detection of neutralizing antibodies to SARS-CoV-2 in human blood, EDTA-plasma or serum. CoNAb is intended for use as an aid in determining whether an individual, either a recipient of a COVID-19 vaccine or a patient recovered from COVID-19 disease, has developed NAb to SARS-CoV-2 or not. The CoNAb Test Kit should not be used to diagnose acute SARS-CoV-2 infection.

PRINCIPLE

CoNAb utilizes the principle of lateral flow or immuno- and protein-chromatography. SARS-CoV-2 spike protein S1 subunit and a tag protein are conjugated to gold nanoparticles (GNP) and embedded in conjugate pad of the device. Recombinant human ACE2 and anti-tag antibody are immobilized on the nitrocellulose membrane to form Test (T) line and Control (C) line, respectively.

The S1 protein and tag protein on GNP can be captured at T line and C line when moving cross nitrocellulose membrane. When NAbs exist in the test sample, they will bind onto S1 proteins on GNP, blocking or neutralizing the interaction between S1 and ACE2, leading to signal at T line diminished or disappeared dependent on the level of NAb in test sample. In contrast, nothing in test sample should interfere the interaction between the tag protein and the anti-tag antibody. The C line will be always show a colored band, serving as a built-in system positive control. The test fails if C line doesn't show up.

PACKAGE

K55102-001 CoNAb Test Kit. All components listed in the table below are packed in a single 4 x 6 inches clear bag.

Components in K55102-001	Quantity
CoNAb Test Kit L55101-001*	1
Pressure Sensitive Lancet	1
Transfer Pipette (20µl)	1
Alcohol pad	1
Micro Sample Buffer Dispenser (0.5ml)	1
Instruction for Use	1
Result Analysis Reference (on the back of clear bag)	1

* L55101-001 contains one CoNAb SARS-COV-2 NAb Test Device and one desiccant bag (1g) sealed in 4 x 3 inches foil bag

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MATERIALS REQUIRED BUT NOT SUPPLIED

Clock or timer, biohazard waste container. disposable gloves, Band-Aid.

STORAGE AND EXPIRATION

1. Store the test kit at 4-30°C. Do Not Freeze.
2. Correctly stored kits are valid for 6 months (See the expiration date indicated on the packaging).
3. The test device should remain in its original sealed pouch until ready for use. After opening, the test device should be used immediately.
4. Do not reuse the device.
5. For long-term storage, refrigeration is recommended.

SPECIMEN COLLECTION AND PREPARATION

1. This test is suitable for human serum, EDTA-plasma, or fingertip blood.
2. Fingertip blood samples must be tested immediately.
3. The test works best on fresh samples. If testing cannot be performed immediately, serum and plasma may be stored at 2-8ºC up to 3 days in case of delay in testing. Avoid repeated freezing/thawing cycles.

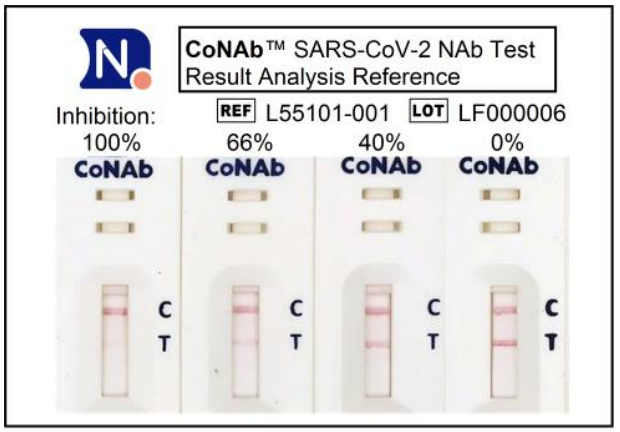
PROCEDURE

1. Read the instructions carefully before use.
2. Bring the kit components to room temperature before testing if the kit was stored in a refrigerator.
3. Open the foil bag and remove all items on a flat surface. Once opened, the test device must be used immediately.
4. If using fingertip sample, clean the collection area with alcohol pad provided. Middle finger or ring finger are recommended.
5. Puncture fingertip using the spring-loaded lancet. To use the lancet, twist its cap **360º** to open it, then push gently against test site until you hear a click. Gently squeeze the finger to help bleeding for sample collection. Sufficient sample amount is critical for sample transferring in next step.
6. Hold the transfer pipette **horizontally without squeezing the pipette**, touch the blood with its tip, wait till blood pass the black line and stop.
7. Transfer the blood to the sample hole of the cassette by squeezing the bulb. Sample won't expel, if the sample does not pass the black line. Please do not try to transfer blood sample if sample amount is not sufficient.
8. Wait for about 20 seconds to allow the blood to settle into sample hole, then **twist** the **white long cap** of the micro sample buffer dispenser **till the rounded tip piece detached** and gently squeeze to add **eight (8)** drops of sample buffer into sample hole. **If the rounded tip piece does not come off, continue to twist it to remove it from the dispenser.**
9. Read result within 15-30 minutes after adding test sample. Do not read result after 30 minutes.
10. If testing serum or plasma in a laboratory, make sure it is prewarmed to room temperature. Use **10µl** of sample for the test.

INTERPRETATION OF RESULTS

A system quality control is included in the test. It will appear red in the Control (C) line if the test has been performed correctly and the reagents are reactive. If the C line doesn't show up, the result is invalid. C line must be positive in any situation.

A lot-specific **Result Analysis Reference** is included with every test (K55102-001) to help users read test result. Additional Control results can be generated by adding Sample Buffer only (without any blood) in a separate CoNAb Test Device. CoNAb is a qualitative lateral flow test that provides a single cutoff at 40% inhibition or neutralization. If the test line shows a color intensity equal to or less than 40%, the individual has developed NAb against SARS-CoV-2. If the T line intensity is stronger than 40%, they either have not developed NAb or limited levels of NAb against SARS-CoV-2, and we recommend confirming NAb status with a quantitative laboratory test (ELISA or PRNT) or consult with a medical doctor. Although a cutoff is set at 40%, it does not imply that there is a significant difference between 39% and 41% or between 35% and 45%. Anyone with a test result that is around 40% should use caution in their daily life to minimize exposure.



Novodiax has evaluated CoNAb and quantitatively determined the approximate detection range with a CE Mark/FDA EUA approved ELISA Test comparator that can be used in CLIA certified professional Laboratories. A complete neutralization (where the Test line completely disappeared) by CoNAb corresponds to positive detection of NAb by comparator after a test sample being a 1:700 or greater dilution. A 50% neutralization by CoNAb equals to about 1:100 by comparison. These dilutions can also be described as NAb titers.

Novodiax monitored NAb level of 14 people who received either Moderna or BioNTech/Pfizer vaccine by CoNAb. Two to 4 weeks after second dose, all 14 people showed more than 75% inhibition with 6 showing 100% inhibition. Then 4-5 months after the second dose, three showed slightly less than 50%, other three were in the range of 50-60%, the rest remained to be more than 75% with two keeping at 100%.

During the development, Novodiax analyzed CoNAb results with a digital reader and the combination of a smart phone camera and professional software. Using such quantitative analytical approaches, the NAb inhibition percentage against the Delta variant showed a median of 1.35 fold (1.07-5.88 folds) reduction compared to that against original COVID-19 virus by CoNAb Test.

PERFORMANCE CHARACTERISTICS

One to ten diluted K2-EDTA plasma samples from 30 RT-PCR positive COVID-19 patients, 8 pre-vaccination persons, 47 COVID-19 vaccine recipients and 80 normal plasmas collected before the COVID-19 pandemic, were tested with CoNAb and compared in parallel with the CE Mark/FDA EUA approved comparison assay. All of the plasma samples from COVID patients were purchased from a reputable biobanking company and collected within 2-4 weeks after COVID-19 symptom onset or during an acute phase infection. All of the plasmas from COVID-19 vaccine recipients were collected 1-23 weeks after receiving second dose. At this dilution, **CoNAb's sensitivity (Positive percent agreement with comparator) is 100% (95% CI: 94.80% - 100%) and specificity (Negative percent agreement with comparator) is 98.85% (95% CI: 93.77% - 99.94%)**.

Clinical agreement with comparator ELISA

		Comparator +	Comparator -	Total
CoNAb	+	70	1	71
	-	0	94	94
	Total	70	95	165

All 80 normal plasmas were collected pre-pandemic between 2014 and 2016 and did not show any detectable neutralizing antibodies against SARS-CoV-2 by either method.

WARNINGS AND PRECAUTIONS

1. A high level of NAb does not guarantee that an individual won't become a virus carrier or spread the virus to other vulnerable people. Excessive fatigue, exposure to high density virus or a highly transmissible variant might overturn the immunity individuals may have built via vaccination or prior infection. Individuals are reminded to manage personal activities and adhere to the rules provided by local public health authorities.
2. The inhibition percentages (or titers) generated by different methods may or may not be comparable or correlated. The only quantitative correlation established for CoNAb is with a CE Mark/FDA EUA approved SARS-CoV-2 neutralizing antibody ELISA.
3. All human samples should be considered and handled as infectious material.
4. K2-EDTA treated plasma is the only plasma tube validated with CoNAb. Labs wishing to use a different plasma tube must perform a separate validation for other types of plasmas.
5. Do not use reagents beyond the stated expiration date.