iFlash-2019-nCoV NAb

REF **C86109** Σ **2×50 Tests** CE

IVD ***In vitro* diagnostic medical device**

For Medical Professional Use Only!

INTENDED USE

The iFlash-2019-nCoV Neutralization Antibody (NAb) assay is a paramagnetic particle chemiluminescent immunoassay (CLIA) for the quantitative determination of 2019-nCoV neutralization antibody in human serum and plasma using the automated iFlash Immunoassay Analyzer. It is intended to be used for evaluation of neutralizing antibodies in patients who had recovered from COVID-19 or monitor neutralization antibodies in people who had COVID-19 vaccination. iFlash-2019-nCoV Neutralization Antibody (Nab) assay is not intended to be used for diagnose or to exclude 2019-nCoV or SARS-COV-2 infection.

SUMMARY AND EXPLANATION

The novel coronaviruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days but still remains infectious during this period. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

ASSAY PRINCIPLE

The iFlash-2019-nCoV Neutralization Antibody assay is a one-step competitive immunoassay using direct chemiluminescence immunoassay.

- 1st incubation: 2019-nCoV NAb in the sample (if exists) reacts with 2019-nCoV Receptor Binding Domian (RBD) antigen-coated paramagnetic microparticles to form a complex.
- 2nd incubation: Acridinium-ester-labeled Angiotensin Converting Enzyme 2 (ACE2) conjugate is added to competitively bind to the RBD-coated particles, which have not been neutralized by the NAb (if exists) from the sample, and form another reaction mixture.
- Washing: Under magnetic field, magnetic particles are adsorbed to the wall of the reaction tube, and unbound materials are washed away by the wash buffer.
- Signal triggering and measuring: The pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs).
- An inverse relationship exists between the amount of 2019-nCoV neutralization antibodies in the sample and the RLUs detected by the iFlash optical system.
- Results are determined via a calibration curve, which is generated by 4-point calibration and a master curve provided via the reagent QR code.

REAGENTS

Reagent kit, 100 tests, 2 packs, 50 tests/pack

R1	2019-nCoV RBD antigen-coated paramagnetic micro-particles, 3.5 mL/pack; 0.05% ProClin 300 as preservative.
R2	Acridinium-ester-labeled ACE2 conjugate; 4.0 mL/pack; 0.05% ProClin 300 as preservative.
R3	Sample treating agent, 6.5 mL/pack; surfactant and 0.05% ProClin 300.
CAL1	Calibrator 1, 1 bottle, 1.0 mL/bottle, 2019-nCoV neutralization antibody in TRIS buffer with protein stabilizer, 0.05% ProClin 300 as preservative.
CAL2	Calibrator 2, 1 bottle, 1.0 mL/bottle, 2019-nCoV neutralization antibody in TRIS buffer with protein stabilizer, 0.05% ProClin 300 as preservative.
CAL3	Calibrator 3, 1 bottle, 1.0 mL/bottle, 2019-nCoV neutralization antibody in TRIS buffer with protein stabilizer, 0.05% ProClin 300 as preservative.
CAL4	Calibrator 4, 1 bottle, 1.0 mL/bottle, 2019-nCoV neutralization antibody in TRIS buffer with protein stabilizer, 0.05% ProClin 300 as preservative.

MATERIALS REQUIRED (BUT NOT PROVIDED)

REF C89999/C89969/C89959/C89949/C89979/C89989, iFlash Pre-Trigger Solution: hydrogen peroxide solution.

REF C89998/C89968/C89958/C89978/C89988/C89948, iFlash Trigger Solution: sodium hydroxide solution.

REF C89997, iFlash Wash Buffer: phosphate buffered saline solution with 0.05% ProClin 300.

REF C80001/C80002, iFlash Wash Buffer (10×): phosphate buffered saline solution with 0.05% ProClin 300.

REF C6100, reaction vessels.

WARNINGS AND PRECAUTIONS

IVD For in vitro diagnostic use only

- No known test method can offer the complete assurance that products derived from human sources will not transmit infection. Therefore, all humanized materials should be considered potentially infectious.
- Due to methodology difference, the use of reagents from diverse manufacturers for sample determination may obtain different results. The results obtained by different reagents cannot be directly compared with each other, so as to avoid wrong medical interpretations.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.

- Wear gloves when handling specimens or reagents.
- Clean and disinfect all spills of specimens or reagents using a suitable disinfectant.
- iFlash Trigger solution contains sodium hydroxide (NaOH) and should be avoided contact with eyes.
- Only authorized personnel who has received official training is permitted for operation.
- This reagent kit is limited to SHENZHEN YHLO BIOTECH CO., LTD. (YHLO) CLIA analyzers (model: iFlash 3000 and iFlash 1800)

REAGENT HANDLING

- The reagents may not be used after the stated expiration date.
- Avoid the formation of foam with all reagents.
- The reagents in the pack are ready for use.
- Close the bottles of calibrator right after calibration and store at 2-8°C.
- Prior to loading the iFlash-2019-nCoV Neutralization Antibody reagent pack on the system for the first time, resuspend the microparticles thoroughly by inverting the reagent pack slightly.
- When reagents are used on the system, the soft caps of reagent bottles must be used to avoid evaporation and pollution. Keep reagents in an upright position.
- To avoid contamination, wear clean gloves when installing soft caps on uncapped reagent bottles.
- Please do not turn over the opened reagent bottle after installing the soft cap to prevent reagent leakage.

STORAGE AND STABILITY

Storage:

- Store at 2-8°C in an upright position.
- The kit may be used immediately after removal from 2-8°C storage.

Stability:

- Unopened at 2-8°C: 6 months.
- Opened at 2-8°C: 28 days.
- Store on-board at 2-8°C: 28 days.
- Production date and expiration date refers to the packaging label.

SPECIMEN COLLECTION AND PREPARATION

- Serum or plasma (lithium heparin, K2-EDTA) are the recommended samples. Other anticoagulants have not been validated for use with the iFlash-2019-nCoV Neutralization Antibody assay.
- Do not use specimens with severe hemolysis and lipemia.
- The assay is affected by icterus (bilirubin>40 mg/dL), hemolysis (Hb>1,000 mg/dL), lipemia (Intralipid> 3,000 mg/dL) total serum protein (> 10 g/dL).
- Do not use specimens with microbial contamination.
- Do not use specimens with clots or flocs.
- Ensure that complete clot formation in serum specimens has occurred prior to centrifugation (No less than 1 hour).
- Centrifuge specimens with a lipid layer on the top, and transfer only the clear specimen without the lipemic material.
- The samples can be tested after one-hour inactivation in a 60°C water bath or oven.
- The samples must be packaged and labeled in accordance with the corresponding national and international regulations on the transportation of clinical samples and infectious materials.
- Specimens should be sent to the laboratory as soon as possible after collection. If specimens need to be transported over long distances, it is recommended to use dry ice and other refrigeration methods for preservation.
- Specimens may be stored at 2-8°C for 3 days at most. For long-term storage, specimens should be kept below -20°C for no more than 90 days.
- Frozen specimens must be mixed thoroughly by vortexing or inverting at low speed after thawing.
- The samples are suggested to be frozen for no more than 3 times.
- Check the samples for air bubbles and remove air bubbles before testing.
- Ensure that the patient samples, calibrators and controls are at ambient temperature (20-25°C) before measurement.
- Due to the possible evaporation, specimens and calibrators on the analyzers should be measured within 2 hours.

ASSAY PROCEDURE

- Refer to the system operating instruction or the online help system for detailed information on preparing the system.
- Prior to loading the iFlash-2019-nCoV Neutralization Antibody reagent pack on the system, the magnetic microparticles (R1) should be fully resuspended.
- The iFlash System will automatically resuspend the magnetic particles before the test.
- The test-specific parameters stored in barcode on the reagent pack are read in. In case the barcode cannot be read, enter the sequence numbers.
- Load samples. The automatic dilution and manual dilution of the iFlash System must match the corresponding detection mode.
- Place the calibrators CAL1, CAL2, CAL3 and CAL4 in the calibrator rack in the sample zone. Only keep calibrators open during calibration.
- The environment temperature during testing is recommended at 10-30°C.
- Calibration application.
- Test application.
- Click RUN, the iFlash System performs all the functions automatically and calculates the results.

CALIBRATION

- Every iFlash-2019-nCoV Neutralization Antibody reagent kit has a QR code label containing the specific information for calibration of the particular reagent lot.
- To perform an iFlash-2019-nCoV Neutralization Antibody calibration, test CAL1. CAL2, CAL3 and CAL4 in duplicate, and the predefined master curve is adapted to the analyzer.
- Once an iFlash-2019-nCoV Neutralization Antibody calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - After 28 days when using the same reagent lot.
  - A reagent kit with a new lot number is used.
  - Controls are out of range.
  - Required by pertinent regulations.

QUALITY CONTROL

Each laboratory should establish its own quality control mean values and acceptable ranges to monitor the test results of this project. Quality control results that do not fall within acceptable ranges may indicate invalid test results and the samples must be tested again. Carry out calibration again, if necessary.

REFERENCE INTERVAL

The iFlash-2019-nCoV Neutralization Antibody reagent pack was analyzed by ROC curve method to determine the cut-off value of 10.00 AU/mL.

INTERPRETATION OF RESULTS

The criteria are as follows:

- Results <10.00 AU/mL is considered as negative. A negative result may indicate absence of SARS-CoV-2 NAb or the level of SARS-CoV-2 NAb is below the limit of detection of this test. A negative result can also be seen when the samples were collected during an acute infection prior to antibody seroconversion.
- Results ≥10.00 AU/mL is considered as positive. A positive result indicates the presence of detectable level of SARS-CoV-2 NAb.

Diagnosis of SARS-CoV-2 infection should be used in conjugation with clinical findings, patient history and other diagnostic procedures.

Results may be affected by following cases:

- Failure to calibrate in accordance with calibration requirements.
- Failure to follow the operating instructions of the applicable instrument.
- The sample collection, preparation, storage, and transportation are not standardized.
- Quality control results are not within the expected ranges.

Confirmation tests are required in the following situations:

- Sample RLU values are different from normal experiments such as too large CV, no RLU values, abnormally high RLU values, etc.
- Results are inconsistent with clinical evidence.

LIMITATIONS

- For medical professional use only.
- This iFlash-2019-nCoV Neutralization Antibody reagent pack is limited to test 2019-nCOV neutralization antibodies in human serum and plasma.
- The use of vacuum blood collector without anticoagulant and blood collectors containing lithium heparin and dipotassium EDTA anticoagulants has been validated for use with this assay.
- The results from an alternative assay (i.e. nucleic acid amplification testing) may not be equivalent and cannot be used interchangeably.
- A negative result can occur if the titer of antibodies against the 2019-nCoV (SARS-CoV-2) virus present in the specimen is below the limit of detection of this assay.
- Positive results may come from current or past infection with some corona virus strains other than 2019-nCoV (SARS-CoV-2), e.g. SARS-CoV-1, MERS, HKU1, NL63, OC43, 229E, etc.
- A positive result may not indicate previous or current 2019-nCoV (SARS-CoV-2) infection. Positive results do not exclude the possibility of re-infection of other strains of SARS-CoV-2.

PHYSICAL PERFORMANCE OF PRODUCTS

Below are the representative performance data, and the results obtained in individual laboratories may differ.

Positive concordance rate

1. The positive concordance rate (+/+) of manufacturer's positive reference materials is 100% (10/10).
2. In a study,iFlash-2019-nCoV NAb assay was used to test 195 people who had vaccinated with 2019-nCoV (SARS-CoV-2) vaccines, 195 people had detectable neutralization antibodies. Positive rate of iFlash-2019-nCoV Nab assay after vaccination is 98.48% (195/198).
3. In a study,iFlash-2019-nCoV NAb assay was used to test 65 patients who had been confirmed as COVID-19 patients by RT-PCR results, 61 patients had detectable neutralization antibodies. Positive rate of iFlash-2019-nCoV NAb assay in COVID-19 patients is 93.8% (61/65).

Negative concordance rate

1. The negative concordance rate (-/-) of the manufacturer's internal negative reference materials is 100% (10/10).
2. In a study,among 270 healthy people who had no COVID-19 infection history and no vaccination history, 268 people were negative for neutralization antibodies. Specificity for iFlash-2019-nCoV NAb assay is 99.26%.

Limit of detection

Use the manufacturer's internal lowest limit of detection reference materials (L1~L4) for testing. L1 should be tested positive, L2 should be tested positive, L3 should be tested positive, and L4 should be tested negative.

Precision

The iFlash-2019-nCoV NAb is designed to have a precision of ≤15% within-laboratory precision.

Analytical Specificity

The analytical specificity of iFlash-2019-nCoV NAb assay was evaluated with antibody-positive samples of patients with H1N1 (new type A H1N1 influenza virus (2009), seasonal H1N1 influenza virus), H3N2, H5N1, H7N9, influenza B Yamagata, Victoria, respiratory syncytial virus, rhinovirus (A, B and C groups), adenovirus (types 1, 2, 3, 4, 5, 7 and 55), enterovirus (A, B, C and D groups), Epstein-Barr virus capsid antigen, Epstein-Barr virus core antigen, measles virus, rubella virus, rotavirus, norovirus, parotid gland Inflammatory virus, varicella-zoster virus, Mycoplasma pneumoniae, Chlamydia pneumoniae, herpes simplex virus type I and herpes simplex virus type II, no cross reactions were found.

The iFlash-2019-nCoV NAb assay is unaffected by HAMA (<600 ng/mL), RF (<1000 IU/mL), ANA (<500 AU/mL) and AMA (＜100 AU/mL).

SHENZHEN YHLO BIOTECH CO., LTD.
Building 1, YHLO Biopark, Baolong 2nd Road, Baolong Subdistrict, Longgang District, 518116 Shenzhen, PEOPLE'S REPUBLIC OF CHINA
E-mail: info@szyhlo.com

EC REP
Lotus NL B.V.
Koningin Julianaplein 10, 1e Verd, 2595AA, The Hague, Netherlands.
E-mail: peter@lotusnl.com

ANNEX A:

Explanation of abbreviation

Abbreviation	Explanation
REF	Catalogue number
CAL	Calibrator
R	Reagent
Σ	Contains sufficient for <n> tests
	Manufacturer
EC REP	Authorized representative in the European Community
CE	CE Conformity Marking
	Caution
	Consult instructions for use
IVD	*In vitro* diagnostic medical device
LOT	Batch code
	Date of manufacture
	Use-by date
2℃ 8℃	Temperature limit (2-8°C)
	Biological risks
GHS07	Pictograms for Caution
GHS09	Pictograms for Hazardous to the aquatic environment
	This way up

ANNEX B:

WARNINGS AND PRECAUTIONS (Proclin 300)

- Hazardous Component: 0.05% Proclin 300 (Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC no. 220-239-6] (3:1))
- Hazard Statement:
  H317: May cause an allergic skin reaction.
  H410: Very toxic to aquatic life with long-lasting effects.
- Precautionary Statement:
  P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
  P272: Contaminated work clothing should not be allowed out of the workplace.
  P273: Avoid release to the environment.
  P280: Wear protective gloves/protective clothing/eye protection/face protection.
  P302+P352: IF ON SKIN: Wash with plenty of soap and water.
  P333+P313: If skin irritation or rash occurs: Get medical advice/attention.
  P321: Seek immediate care from a doctor.
  P363: Wash contaminated clothing before reuse.
  P391: Collect spillage.
  P501: Dispose of contents/container in a safe way.