NanoEntek

CE IVD

FREND™ FLU A&B

Qualitative assay for influenza A and B strain

REF FRFL 020

IVD For *in vitro* diagnostic use only

Intended use

The FREND™ FLU A&B is designed for the qualitative measurement of Influenza A virus and Influenza B virus in human nasopharyngeal swab specimens by fluorescence immunoassay (FIA) using the FREND™ System.

Principle of the assay

A specimen (stored in VTM) is added to a specimen extraction tube and mixed. A well-mixed sample 35 μL is transferred to the sample inlet of a single use FREND™ FLU A&B cartridge. The cartridge is then placed into the FREND™ System, which is programmed to begin analysis once the sample has reacted with the reagents. The reaction and analysis time is approximately 4 minutes. The anti-Influenza A virus and Influenza B virus qualitative measurement is based on the ratio of fluorescence detected by the FREND™ System at the FREND™ Test and Reference zones. The magnitude of the fluorescent ratio is proportional to the presence and absence of Influenza A virus and Influenza B virus in the sample.

Material provided

Q'ty	Contents	Catalogue number
20	Cartridges	FRFL 020
20	Specimen extraction tubes	
20	Filter cap tubes	
30	Disposable pipette tips	
20	Disposable sterile swab	
01	Code chip	
01	Package insert	

Warning and Precautions

- The FREND™ FLU A&B cartridges are intended for *in vitro* diagnostic use only.
- The FREND™ FLU A&B cartridges are only to be used on the NanoEntek FREND™ System.
- The FREND™ FLU A&B cartridges and specimen extraction tubes are disposable, single use devices. Do not reuse them under any circumstances.
- Allow sealed cartridges to come to room temperature for 15-30 minutes prior to use.
- Cartridges and specimen extraction tubes should not be frozen.
- Assure the humidity in the laboratory is in the 10-80% range when tests are run.
- Avoid cross-contamination between samples by using a new pipette tip for each new specimen.
- Avoid high humidity, direct sunlight or heat when storing cartridges and specimen extraction tubes.
- Testing of contaminated samples may cause erroneous results.
- Over or under loading the cartridge with sample may result in inaccurate results.
- Do not use the cartridges beyond the expiration date on the pouch.
- Do not use the cartridge if the pouch is damaged or the seal is broken.
- Perform testing as specified in the Package insert and User manual.
- Keep the cartridge sealed in the pouch until ready for use.

- Use the cartridge immediately after opening the pouch.
- For professional use only.
- Human specimens are not used in the preparation of this product, however, since human specimens will be used for samples and other quality control products in the laboratory may be derived from human materials, Use Universal Precautions when handling all specimens and controls. Wear disposable gloves when handling the cartridges and the samples.
- Wash hands thoroughly and often after handling reagent cartridges or samples.
- Do not ingest the silica gel packet found in the cartridge pouch.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at refrigerator temperature storage (2-8℃). Reagent stability has been demonstrated for twelve months from the date of manufacture.
The expiration date is clearly indicated on the product box and the cartridges.

Specimen collection and handling

To collect nasopharyngeal swab specimens, insert the sterile swab into the nostril that presents the most secretion under visual inspection. Using gentle rotation push the swab until resistance is met at the level of the turbinate(less than one inch into the nostril). Rotate the swab a few times against the nasal wall.

It is recommended to use samples immediately. However, if testing is not done immediately, samples may be stored VTM at 2-8℃ for up to 3 days prior to testing. If testing is not performed within 3 days, store at -70℃ or below. Samples can be stored frozen for up to 12 months prior to testing.

Repeated freeze-thaw cycles should be avoided. Prior to assay, slowly bring frozen samples to room temperature and mix gently but thoroughly before testing.

When pipetting into the cartridge sample inlet, ensure that bubbles in the sample are avoided. Bubbles may restrict flow and result in an incomplete or erroneous test result.

Procedure

Code chip installation

A lot-specific Code chip is supplied with each kit of FREND™ FLU A&B. When using a new lot of reagent, the Code chip of the same lot must be installed in the FREND™ System. Please refer to the FREND™ System User manual for more detailed instructions relative to the Code chip installation. Abbreviated instructions are as follows:

(1) Insert the FREND™ System electrical cord into an appropriate outlet.
(2) Insert the Code chip into the Code chip slot at the rear of the system following the arrows.
(3) Press the 'Setup' button on the 'Main' screen.
(4) Press the 'Code chip' button on the 'Setup' screen.
(5) The information embedded on the FREND™ FLU A&B Code chip is automatically saved on the FREND™ System.
(6) When the Code chip installation is completed, press the 'OK' button to go to the 'Setup' screen.
(7) Press the 'Item' button on the 'Setup' screen.
(8) Check the FREND™ FLU A&B cartridge lot number and the installation date of the Code chip.
(9) Press the 'Home' button to go to the 'Main' screen to begin running external quality control and patient samples.

Specimen processing

Allow the sealed pouches containing the FREND™ FLU A&B cartridges and specimen extraction tubes to come to room temperature for 15-30 minutes prior to use.
If using refrigerated patient samples, remove those from the refrigerator and allow them to come to room temperature prior to testing. If frozen samples will be utilized, be sure these are removed from the freezer, thawed naturally and then mix gently but thoroughly prior to testing. Testing should not begin on frozen samples until they have reached room temperature.

Assay procedure

(1) Insert the patient sample swab into extraction tube and swirl the swab 10 times.
(2) Remove the swab while pressing the swab head against tube to extract liquid from the swab.
(3) Discard the used swab.
(4) Close the tube with filter cap tube.
(5) Squeeze the specimen extraction tube from the top with the filter cap tube at the bottom.
(6) Remove the specimen extraction tube from the filter cap tube.
(7) Using the micropipette, drop 35 μL of the sample in the filter cap tube onto the cartridge inlet.
(8) Press the 'Test' button on the 'Main' screen of the FREND™ System.
(9) The system moves to the Patient ID screen automatically.
(10) Type the Patient ID and press the 'Enter' button to begin the test.
(11) Insert the cartridge into the cartridge slot using the cartridge arrows as a guide.

⚠ ***Caution:*** *Please check the direction of the cartridge before insertion and assure the insertion is complete.*

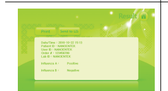
⚠ ***Caution:*** *Please insert the cartridge into the FREND™ System after loading the sample cartridge.*

(12) When the reaction in the cartridges is complete, the FREND™ System will automatically begin the reading.
(13) When the reading has been completed, the cartridge will automatically be expelled and the results displayed.

⚠ ***Caution:*** *Do not remove power from the FREND™ System while a cartridge is in the reading chamber.This may cause a system error.*

(14) If the FREND™ System is connected to the optional printer, press the 'Print' button and the results will be output on the printer paper.
(15) For more detailed instructions, please refer to the 'FREND™ System User manual'.

Display of Results

Displayed result	Description
	Influenza A "Negative" Influenza B "Negative"
	Influenza A "Positive" Influenza B "Negative"
	Influenza A "Negative" Influenza B "Positive"
	Influenza A "Positive" Influenza B "Positive"

Interpretation of results

Influenza A Positive result

A positive result on the presence of nucleoprotein from influenza A strain, but it does not rule out co-infections with other pathogens.

Influenza B Positive result

A positive result on the presence of nucleoprotein from influenza B strain, but it does not rule out co-infections with other pathogens.

Negative result

Negative result on the presence of influenza A and B nucleoproteins, but it does not rule out false negative.

Performance evaluation

1) LoD (Limit of Detection, LoD)

Sample type	LoD (Limit of Detection)
Influenza Antigen A/Texas/36/91	5 ngHA/mL
Influenza Antigen B/Panama/45/90	11 ngHA/mL

2. Interference
In the FREND™ FLU A&B test, it was confirmed that the following interference were not affected.

NO.	Substances	Concentration
1	Whole Blood (Type A)	4%
2	Whole Blood (Type B)	4%
3	Whole Blood (Type AB)	4%
4	Whole Blood (Type O)	4%
5	Mucin from bovine submaxillary gland	1 μg/mL

6	4-Acetamidophenol	1 mg/mL
7	Acetylsalicylic acid	1 mg/mL
8	Albuterol	0.083 mg/mL
9	Amantadine Hydrochloride	900 ng/mL
10	Afrin (Oxymetazoline)	50 μg/mL
11	Beclomethasone	500 ng/mL
12	Budesonide	500 ng/mL
13	Benzocaine	1.5 mg/mL
14	Chlorpheniramine maleate	5 mg/mL
15	CVS Nasal Drops (Phenylephrine)	1 mg/mL
16	CVS Nasal Spray (Cromolyn)	1 mg/mL
17	Dexamethasone	2.5 mg/mL
18	Diphenhydramine HCl	5 mg/mL
19	Fexofenadine	500 ng/mL
20	Fluticasone	500 ng/mL
21	Guaiacol Glyceryl Ether (guaifenesin)	5 mg/mL
22	Ibuprofen	500.3 μg/mL
23	L-ascorbic acid	29.9 μg/mL
24	Loratidine	100 ng/mL
25	Mometasone	500 ng/mL
26	Mupirocin	1 mg/mL
27	Oseltamivir Phosphate	500 ng/mL
28	Pseudoephedrine HCl	2 mg/mL
29	Tobramycin	500 ng/mL
30	Triamcinolone	500 ng/mL
31	Zanamivir	1 mg/mL
32	HALLS honey lemon	5 %(w/w)
33	Solar C	1 %(w/w)
34	CoN	5 %(w/w)
35	MoKN	5 %(w/w)
36	Listerine cool mint	5 %(w/w)
37	Galphimia glauca	10 mg/mL
38	Histaminum hydrochloricum	10 mg/mL
39	Flunisolide	250 μg/mL
40	Sodium Chloride with Preservatives	0.06 %

3. Cross-reactivity
The following substances were evaluated for potential cross-reactivity with FREND™ FLU A&B. No significant cross-reactants was found itself.

NO.	Substances (Virus)	Concentration (PFU/mL)
1	Coxsackievirus B5	1 X 10^6
2	Echovirus 9	1 X 10^6
3	Human adenovirus B (Adenovirus type 3)	1 X 10^5
4	Human adenovirus B (Adenovirus type 11)	1 X 10^6
5	Human adenovirus C (Adenovirus type 1)	1 X 10^6
6	Human adenovirus C (Adenovirus type 5)	1 X 10^6
7	Human adenovirus E (Adenovirus type 4)	1 X 10^6
8	Human coronavirus (229E)	1 X 10^5
9	Human corona virus Betacoronavirus 1 (OC43)	1 X 10^6
10	Human cytomegalovirus (Human herpesvirus 5)	1 X 10^6
11	Human enterovirus A (Enterovirus Type 71)	1 X 10^5
12	Human enterovirus D (Enterovirus Type 70)	1 X 10^5
13	Human Rhinovirus A, Human Rhinovirus 7	1 X 10^5
14	Human Rhinovirus A, Human Rhinovirus 8	1 X 10^6
15	Human Rhinovirus B, Human Rhinovirus 14	1 X 10^5
16	Human Rhinovirus B, Human Rhinovirus 42	1 X 10^5
17	Measles virus	1 X 10^5
18	Mumps virus	1 X 10^6
19	Parainfluenza virus 1	1 X 10^5
20	Parainfluenza virus 2	1 X 10^6
21	Parainfluenza virus 3	8 X 10^5
22	Parainfluenza virus 4a	1 X 10^5
23	Parainfluenza virus 4b	1 X 10^5
24	Respiratory syncytial virus A	1 X 10^6
25	Respiratory syncytial virus B	1 X 10^5
26	Respiratory syncytial virus A2	1 X 10^5

NO.	Substances (Bacteria)	Concentration (CFU/mL)
1	Candida albicans	1.0X 10^6
2	Corynebacterium diphtheriae	1.0 X 10^6
3	Enterococcus faecalis	2.0 X 10^7
4	Enterococcus faecium	1.25 X 10^6
5	Haemophilus influenzae	1.0 X 10^6
6	Klebsiella pneumoniae subsp. Pneumoniae	1.0 X 10^6
7	Moraxella catarrhalis	1.25 X 10^6
8	Neisseria meningitidis	1.0 X 10^6
9	Pseudomonas aeruginosa	1.0 X 10^7
10	Serratia marcescens	5.0 X 10^6
11	Staphylococcus aureus subsp	1.0 X 10^7
12	Staphylococcus epidermidis	1.0 X 10^7
13	Streptococcus agalactiae(ATCC® 12403™)	5.0 X 10^6
14	Streptococcus agalactiae (ATCC® 27956™)	1.0 X 10^6
15	Streptococcus agalactiae Lehmann and Neumann (Group A Streptococcus)	1.0 X 10^6
16	Streptococcus dysgalactiae subsp.	1.0 X 10^7
17	Streptococcus dysgalactiae subsp. equisimilis (Group A Streptococcus)	1.0 X 10^6
18	Streptococcus equi subsp.	2.0 X 10^6
19	Streptococcus pyogenes Rosenbach	1.25 X 10^6
20	Streptococcus salivarius subsp.	1.0 X 10^6

4. Precision
As a result of the repeated and reproducible test for FREND™ FLU A&B, all negative samples were negative, and all positive samples were positive, which met the criteria.

5. Clinical performance
The total of 411 clinical specimens (195 influenza A positive, 85 influenza B positive, and 131 negative) confirmed with RT-PCR were tested with the FREND™ FLU A&B.

(1) Influenza A result

Method		RT-PCR	
		Positive	Negative
FREND™ FLU A&B (Influenza A)	Positive	164	0
	Negative	31	131
Total		195	131

- Sensitivity: 84.10% (164/195) (95% CI: 78.32% ~ 88.57%)
- Specificity: 100% (131/131) (95% CI: 97.15% ~ 100%)
- Positive Predictive Value (PPV): 100% (164/164) (95% CI: 97.71% ~ 100%)
- Negative Predictive Value (NPV): 80.86% (131/162) (95% CI: 74.12% ~ 86.18%)

(2) Influenza B result

Method		RT-PCR	
		Positive	Negative
FREND™ FLU A&B (Influenza B)	Positive	74	0
	Negative	11	131
Total		85	131

- Sensitivity: 87.06% (74/85) (95% CI: 78.30% ~ 92.62%)
- Specificity: 100% (131/131) (95%CI: 97.15% ~ 100%)
- Positive Predictive Value (PPV): 100% (74/74) (95% CI: 95.07% ~ 100%)
- Negative Predictive Value (NPV): 92.25% (131/142) (95% CI: 86.66% ~ 95.62%)

6. Agreement
The total of 411 clinical specimens (195 influenza A positive, 85 influenza B positive and 131 negative) confirmed with Predicate device were tested with the FREND™ FLU A&B.

(1) Influenza A result

Method		RT-PCR	
		Positive	Negative
FREND™ FLU A&B (Influenza A)	Positive	164	0
	Negative	15	147

- Total Agreement: 95.40% (95% CI: 92.55% ~ 97.19%)
- Positive Percent Agreement: 91.62% (95% CI: 86.64% ~ 94.86%)
- Negative Percent Agreement: 100% (95% CI: 97.45% ~ 100%)
- Cohen's kappa = 0.9084

(2) Influenza B result

Method		RT-PCR	
		Positive	Negative
FREND™ FLU A&B (Influenza B)	Positive	74	0
	Negative	3	139

- Total Agreement: 98.61% (213/216) (95% CI: 96% ~ 99.53%)
- Positive Percent Agreement: 96.10% (74/77) (95% CI: 89.16% ~ 98.67%)
- Negative Percent Agreement: 100% (139/139) (95% CI: 97.31% ~ 100%)
- Cohen's kappa = 0.9745

Glossary of symbols

	Caution, warning, Consult accompanying documents
	Catalogue number/Reference number
	Lot number/Batch number
	Use by YYYY-MM-DD or YYYY-MM
	Manufacturer
	Authorized representative in the European Community
	CE marking
	*In vitro* diagnostic medical device
	Temperature limitation
	Contains sufficient for <n> tests
	Do not reuse
	Do not use if package is damaged
	For prescription use only
	Irritant

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