INTENDED USE
To detect influenza A virus antigens and influenza B virus antigens in nasal aspirate, nasal swab, nasal discharge/nasal mucus or pharyngeal swab (to assist in the diagnosis of influenza virus infectious disease).

SUMMARY AND EXPLANATION OF THE TEST
Influenza spreads around the world in seasonal epidemics, resulting in about 3 to 5 million annual cases of severe illness and about 250,000 to 500,000 annual deaths, rising to millions in some pandemic years. (WHO News Release, 2017)

Common symptoms are chills, fever, sore throat, muscle pains, headache, coughing, weakness/fatigue and general discomfort.

Diagnosis is difficult because the initial symptoms can be similar to those caused by other infectious agents. The influenza A virus is usually more prevalent and is associated with the most serious influenza epidemics, while influenza B infections usually present more mild symptoms.

Because the influenza virus is highly contagious, rapid diagnosis and prompt treatment can have a positive effect on public health. And the ability to distinguish between A or B antigens can help the physician to prescribe an appropriate antiviral therapy.

Administration of antiviral therapy within 48 hours of symptom onset is recommended for more rapid reduction of symptoms and to reduce viral shedding.

Capilia Flu Neo can provide rapid and accurate detection of influenza A and/or B virus antigens from symptomatic patients. No special instruments or equipment are required.

PRINCIPLE OF THE TEST
Measurement using this product is based on an immunochromatography assay using a monoclonal antibody that recognizes influenza virus antigens. This product comprises a test plate with a carrier strip containing a sample placement area, a reagent area including a colloidal platinum-gold labeled anti-influenza A and B virus monoclonal antibody (mouse) (hereinafter referred to as “colloidal platinum-gold labeled antibody”), a reading area [A] that fixes the anti-influenza A virus monoclonal antibody (mouse) (hereinafter referred to as “anti-influenza A virus antibody”), a reading area [B] that fixes the anti-influenza B virus monoclonal antibody (mouse) (hereinafter referred to as “anti-influenza B virus antibody”), and a reading area [C] that fixes the anti-mouse immunoglobulin polyclonal antibody (rabbit) (hereinafter referred to as “anti-mouse immunoglobulin antibody”).

When a sample is placed on the sample placement area of the test plate, the colloidal platinum-gold labeled antibody dissolves and forms an immune complex with the influenza A and/or B virus antigens in the sample. This immune complex migrates through the developing area by capillary action, is captured by the anti-influenza A virus antibody and/or the anti-influenza B virus antibody fixed in the developing area, and forms a black line in the reading area [C]. This means the colloidal platinum-gold labeled antibodies have migrated normally.

REAGENTS AND MATERIALS PROVIDED
Capilia Flu Neo (20 Tests)
Test plates
- Components
  - Colloidal platinum-gold labeled anti-influenza A, B virus monoclonal antibody (mouse)
  - Anti-influenza A virus monoclonal antibody (mouse)
  - Anti-influenza B virus monoclonal antibody (mouse)

Extraction Buffer (to be used equally with the four products).
- Components
  - Buffer, detergent, sodium azide (0.09%)

Nozzles
Capilia Flu Neo (10 Tests)
Test plates
- Components
  - Colloidal platinum-gold labeled anti-influenza A, B virus monoclonal antibody (mouse)
  - Anti-influenza A virus monoclonal antibody (mouse)
  - Anti-influenza B virus monoclonal antibody (mouse)

Extraction Buffer (to be used equally with the four products).
- Components
  - Buffer, detergent, sodium azide (0.09%)

The extraction buffer is able to be used equally with the four products below:
- Capilia Flu Neo (rapid test for detecting influenza virus antigen)
- Capilia Adeno Neo (rapid test for detecting adenovirus antigen)
- Capilia RSV Neo (rapid test for detecting RS virus antigen)
- Capilia hMPV (rapid test for detecting human metapneumovirus antigen)

 MATERIALS REQUIRED BUT NOT PROVIDED
Capilia Flu Neo (20 Tests)
Timer, micropipette, pipette tips, suction machine, suction trap, the specimen collecting sheet for nasal discharge, sterile swabs (as listed here)

Recommended Swab
The following swabs are recommended for use with the kit.

For Nasal Swab
1. FLOQSswabs ™ (Cat No. 534CS01, Copan Italia S.p.A, Italy)
2. Sterilized Swab P165A 10 pcs (Cat No. 4124, HEIWA MEDIC. CO., LTD, Japan)

For Pharyngeal Swab
1. FLOQSswabs ™ (Cat No. 502CS01, Copan Italia S.p.A, Italy)
2. Sterilized Swab PL6S 10 pcs (Cat No. 4371, HEIWA MEDIC. CO., LTD, Japan)

To collect nasal aspirate, any of the above swabs may be used.
Acceptable Swab
The following swabs are acceptable for use with the kit.
- Tip material
  - Rayon, flocked nylon and polyester
- Standard tip size
  - For nasal swab
    - Plain dry swab: maximum diameter 3 mm, length 12 mm
    - Flocked swab: maximum diameter 3 mm, length 15 mm
  - For pharyngeal swab
    - Plain dry swab: maximum diameter 6 mm, length 14 mm
    - Flocked swab: maximum diameter 6 mm, length 16 mm
- Shaft material
  - Paper, plastic (PS, nylon), aluminum

Unacceptable Swab
Do not use calcium alginate swabs.

**WARNIMG AND PRECAUTIONS**

1. Precautions when handling (including hazard control)
1) All specimens should be handled as potentially infectious, and special precautions should be exercised.
2) In consideration of the risk of infection, wear protective clothes such as a mask and gloves and handle the specimens and samples carefully during the test.
3) If the extraction buffer gets into your eyes, immediately flush with a large quantity of water for 15 minutes or more. If you still feel some abnormality, see a doctor for treatment.
4) If the extraction buffer comes into contact with your hands or clothes, wash your hands and/or clothes with soap and a large quantity of water.
5) To be handled by trained personnel only.
6) Swabs should not be used if the swab package is open or damaged and precautions should be exercised.
7) Do not use a swab if it is broken, bent or stained.
8) Avoid the following when using the swab since they may cause the breakage of the shaft.
   - To use the swab in a manner such that the shaft (especially the narrow part of the shaft) receives too much force, pressure or torsional load at the time of specimen collection.
   - To intentionally deform the shaft, more specifically, to bend, arch or break the shaft.
9) Particularly when collecting a pharyngeal swab from a child, collect the specimen with great caution lest he or she should act up, bite or break the swab and swallow the broken part, and by firmly retaining the upper body of the child to be tested

2. Precautions when using
1) This product reacts only with influenza A and B viruses and does not react with C virus.
2) This product is a rapid test for detecting influenza A and B virus antigen. A definite diagnosis should be made by an attending physician, in combination with the clinical symptoms, the result of viral isolation culture test and other test results.
3) This product should be used in accordance with the procedure stated in the package insert.
4) In order to prevent deterioration, this product should be stored between 2°C and 30°C, avoiding high temperatures, high humidity and direct sunlight.
5) If this product has been refrigerated, it must be removed from the refrigerator at least 30 minutes before use and kept at room temperature when used for testing.
6) The aluminum pouch containing a test plate should not be opened until the test plate is about to be used.
7) The sample placement area and the reading area of the test plate should not be touched with the hands.
8) A precipitate may be seen in the extraction buffer, but the product can be used as it is, because the precipitate has been shown not to affect test results.
9) For nasal sampling, do not keep forcibly insert the swab, when the distance to the site is clearly shorter than usual. In particular, there is the possibility of resistance being imposed on the stick when the sample is collected from an infant or a patient with a narrow nasal cavity. In such a case, do not swab hard, exerting force on the stick. Moreover, do not rotate the stick forcibly.
10) For nasal sampling, any mass of mucus on the tip of the swab should be gently removed with gauze. Do not wipe the tip too hard. Epithelial cells should remain on the tip for testing.
11) When sampling a nasal discharge, be careful not to spill or scatter the samples, as it may cause a secondary infection.
12) Do not use any products beyond the expiration date.

3. Precautions when using swabs
1) Use the swab provided in the kit according to the following instructions:
   - Open the peel package from the side indicating "PEEL HERE." and pull the swab out.
   - Peel the film, then remove the swab by pinching the shaft, avoiding contact with the swab tip, and directly use the swab for collecting the specimen.
2) A swab is for single use only; reuse is not allowed in any case. Reuse may cause a risk of infection and/or inaccurate results.
3) Do not re-sterilize unused swabs.
4) Do not re-pack.
5) To be handled by trained personnel only.
6) Swabs should not be used if the swab package is open or damaged and collect samples immediately after opening.
7) Do not use a swab if it is broken, bent or stained.
8) Avoid the following when using the swab since they may cause the breakage of the shaft.
   - To use the swab in a manner such that the shaft (especially the narrow part of the shaft) receives too much force, pressure or torsional load at the time of specimen collection.
   - To intentionally deform the shaft, more specifically, to bend, arch or break the shaft.
9) Particularly when collecting a pharyngeal swab from a child, collect the specimen with great caution lest he or she should act up, bite or break the swab and swallow the broken part, and by firmly retaining the upper body of the child to be tested.

4. Precautions for disposal
1) Because used test plates, swabs, tubes and nozzles after use, remaining samples, etc. may cause infections, they should be autoclaved (121°C, 20 min) or soaked in 0.1% sodium hypochlorite for more than one hour. When remaining reagents or their accessories are disposed of, they should be treated in accordance with the laws and regulations concerning medical waste disposal and water pollution control.
2) In the extraction buffer, 0.09% of sodium azide is included as a preservative. When solutions containing sodium azide continue to be discarded over a long period of time, explosive metallic azide may be produced if a drain is made of metal. Therefore, they should be discarded with a large quantity of water.

**STORAGE CONDITIONS**
Storage : Store at 2°C to 30°C. DO NOT FREEZE.
Keep away from direct sunlight.
Do not use test plate or extraction buffer after expiration date.

**SPECIMEN COLLECTION AND PREPARATION**

1. Methods of specimen collection

1) Sampling of nasal aspirate
   - Firmly insert one tube of the suction trap into the suction pump, and the other tube into a nasal cavity through an external nostril. Collect the nasal discharge aspirate in the suction trap by operating the suction pump. Soak a swab in the nasal aspirate collected by the trap, and let the swab absorb the nasal aspirate well. When nasal aspirate is taken using a micropipette or other instruments, dilute the nasal aspirate twofold with physiological saline and sample 200 μL of this dilution.

2) Sampling of nasal swab
   - Firmly insert a nasal swab into the nasal cavity and collect mucosal epithelium by swabbing the nasal turbinate several times.

3) Sampling of pharyngeal swab
   - Firmly insert a pharyngeal swab into the pharynx through the oral cavity, and collect the mucosal epithelium by swabbing the posterior wall of the pharynx and the palatine tonsil several times, centering around the rubefacient portion. Avoid touching saliva.
   - If the specimen is mixed with saliva, the lines on the test plate may become fainter.

4) Sampling of nasal discharge/nasal mucus
   - When the patient’s condition is judged to be suitable for collecting nasal discharge, the patient should blow his/her nose using the specimen collecting sheet. Swab the collected nasal discharge with a nasal swab. Or directly swab the nostril to obtain the specimen.
   - If either sampling method collects insufficient samples, try other methods.

With a collected specimen, prepare the sample and perform the test as quickly as possible.
2. Sample preparation

Remove the aluminum sealing cap from the extraction buffer tube, while taking care not to spill the liquid.

Soak the swab that collected the specimen in the extraction buffer, and stir well. Then, pinch the tip of the swab firmly with the soft wall of the extraction buffer tube with your fingers and squeeze out the swab. Use this squeezed-out liquid as the sample. When using a nasal aspirate specimen diluted twofold with physiological saline, add 200 μL of the specimen to the extraction buffer in the tube, and mix well. Use this mixture as the sample.

3. Precautions for sample preparation

For a highly viscous sample that can cause filter clogging, dilute the sample twofold with physiological saline before use.

**TEST PROCEDURE**

1. Firmly attach the nozzle (with a filter) provided in the kit to the top of the extraction buffer tube.
2. Hold the middle of the tube with the fingers and dispense 3 drops of the sample (80–120 μL) onto the sample placement area of the test plate. Hold the tube perpendicularly and take care not to let the tip of the nozzle touch the sample placement area.
3. Observe the reading area of the test plate after 3 to 5 minutes for a positive result and after 5 minutes for a negative result, and interpret the result according to the "READING TEST RESULTS:"

**Precautions for sample placement**

If an excessive amount of the sample is dispensed, the reaction time may be prolonged due to the dilution of the colloidal platinum-gold labeled antibody based on the principle of the test, and no line may be seen at [C] and/or [T] in the reading area within the judgment time or the line is faint.

**READING TEST RESULTS**

Allow the samples to react according to the test procedure and read the black lines that appear in the reading area.

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-positive</td>
<td>When black lines are seen at both [A] and [C] in the reading area (two lines), the result is read as positive for influenza A virus antigen. When a very faint black line is seen in the reading area [A], the result is interpreted as positive.</td>
</tr>
<tr>
<td>B-positive</td>
<td>When black lines are seen at both [B] and [C] in the reading area (two lines), the result is read as positive for influenza B virus antigen. When a very faint black line is seen in the reading area [B], the result is interpreted as positive.</td>
</tr>
<tr>
<td>Negative</td>
<td>When no black line is seen at [A] and [B] in the reading area but a black line is seen only at [C] in the reading area (one line), the result is read as negative. When the line at [C] in the reading area is faint but visually recognizable, chromatographic development has occurred normally.</td>
</tr>
</tbody>
</table>

Retesting

When no black line is seen at [C] in the reading area, there may be some problem with the test procedure or the reagent quality. The test should be performed again, using another test plate.

A line that appears anywhere within the sections of the reading area, which are separated by color, is considered valid.

(NOTE)

1. When black lines are seen at both [A] or [B] and [C] in the reading area 3 to 5 minutes after dispensing the sample, the result is read as A-positive or B-positive. No black line at [A] and [B] in the reading area even 5 minutes after dispensing the sample indicates a negative result.
2. Do not use the test plate for reading a result beyond the judgment time as the result may change due to drying, etc.
3. If an excessive amount of the sample is dispensed, the reaction time may be prolonged due to the dilution of the colloidal platinum-gold labeled antibody based on the principle of the test, and no line may be seen at [C] and/or [T] in the reading area. In that case, dilute the sample with more extraction buffer and perform the test again.
4. If the amount of antigen is very high, a very thick line may be seen at [A] or [B] in the reading and no black line may be seen at [C] in the reading area. In that case, dilute the sample with more extraction buffer and perform the test again.
5. The line is valid even if there is unevenness in depth and there are breaks in the line.

**LIMITATIONS**

1. This product is a rapid test for detecting influenza A and B virus antigen. A definite diagnosis should be made by an attending physician, in combination with the clinical symptoms, the result of viral isolation culture test and other test results.
2. If a pharyngeal swab, nasal discharge or nasal mucus is used as a specimen, the result may be affected by the presence of nasal flora. Therefore, please use a nasal swab, nasal aspirate, or pharyngeal swab as a specimen.
3. The test plate should be used immediately after opening the packaging. When it absorbs moisture, the quality deteriorates and an accurate result cannot be obtained.
4. This product should be used for in vitro diagnosis only and should not be used for any other purposes.
5. Please use this product following the operational method described in this package insert. We cannot guarantee results obtained from any other operations and for any other purposes that are not described in the package insert.
6. The extraction buffer contains sodium azide. If the solution comes into contact with the eye or mouth or adheres to the skin by mistake, take emergency measures such as thorough washing with water and seek medical treatment, if necessary.

**PERFORMANCE CHARACTERISTICS**

1. **Clinical data**

   The result of the clinical performance evaluation in Japan (Comparison with isolation culture method).

   Nasal swab, nasal aspirate, pharyngeal swab : Exams carried out in the flu season in 2006 to 2007.

   Nasal discharge/nasal mucus : Exams carried out in 2008.

   **Table: Clinical data (Comparison with isolation culture method)**

<table>
<thead>
<tr>
<th>Kind of samples</th>
<th>Type</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Accuracy (%)</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal swab</td>
<td>Type A</td>
<td>94.3 (100/106)</td>
<td>95.4 (187/196)</td>
<td>95 (287/302)</td>
<td>302</td>
</tr>
<tr>
<td>Type B</td>
<td>100 (69/69)</td>
<td>98.7 (230/233)</td>
<td>99 (299/302)</td>
<td>302</td>
<td></td>
</tr>
<tr>
<td>Nasal aspirate</td>
<td>Type A</td>
<td>95.1 (58/61)</td>
<td>98.4 (188/191)</td>
<td>97.6 (246/252)</td>
<td>252</td>
</tr>
<tr>
<td>Type B</td>
<td>100 (61/61)</td>
<td>100 (191/191)</td>
<td>100 (252/252)</td>
<td>252</td>
<td></td>
</tr>
<tr>
<td>Pharyngeal swab</td>
<td>Type A</td>
<td>87.3 (55/63)</td>
<td>94.8 (128/135)</td>
<td>92.4 (183/198)</td>
<td>198</td>
</tr>
<tr>
<td>Type B</td>
<td>91.5 (75/82)</td>
<td>99.1 (115/116)</td>
<td>96 (190/198)</td>
<td>198</td>
<td></td>
</tr>
<tr>
<td>Nasal discharge/nasal mucus</td>
<td>Type A</td>
<td>84.1 (53/63)</td>
<td>96.9 (94/97)</td>
<td>91.9 (147/160)</td>
<td>160</td>
</tr>
<tr>
<td>Type B</td>
<td>96.2 (50/52)</td>
<td>98.1 (106/108)</td>
<td>97.5 (156/160)</td>
<td>160</td>
<td></td>
</tr>
</tbody>
</table>

2. **Sensitivity (Detection limit)**

   The minimum detection limit is 7.5 × 10^{3} TCID_{50}/test for the influenza A virus antigen and is 7.5 × 10^{3} TCID_{50}/test for the influenza B virus antigen.
3. Reactivity

Reactivity was found in the following strains:

1) Human origin type A virus
   - A/New Jersey/8/76 (H1N1)
   - A/Sendai/782/06 (H1N1)
   - A/Sendai/197/07 (H1N1) A/Adachi/1/57 (H2N2)
   - A/Sendai/F492/06 (H3N2)
   - A/Sendai/958/07 (H3N2) A/Aichi/2/68 (H3N2)
   - A/Anhui/1/2013 (H7N9)

2) Human origin A(H1N1)pdm09
   - A/Osaka/50/09
   - A/Osaka/56/09
   - A/Osaka/60/09
   - A/Osaka/65/09
   - A/Osaka/71/09
   - A/Osaka/84/09
   - A/Osaka/100/09
   - A/Osaka/104/09
   - A/Osaka/108/09
   - A/Osaka/114/09
   - A/Osaka/119/09
   - A/Osaka/143/09
   - A/Osaka/157/09
   - A/Osaka/168/09
   - A/Osaka/174/09

3) Type A virus of other than human origin
   - A/duck/Tottori/72/80 (H1N1)
   - A/duck/Mongolia/403/98 (H3N8)
   - A/chicken/Yamaguchib/7/04 (H7N7)
   - A/whooper swan/Mongolia/305/HSN1
   - A/duck/Hong Kong/280/80 (H5N3)
   - A/shearwater/Australiab/17/HSN5
   - A/chicken/Pakistan/447/95 (HS73)
   - A/chicken/Netherlands/2586/03 (HS77)
   - A/duck/Mongolia/19/2008 (HS79)
   - A/duck/Hokkaido/17/01 (HS73)
   - A/duck/Czechoslovakia/1/56 (HS66)
   - A/duck/Pennysylvania/10218/84 (HS52)
   - A/duck/Massachusetts/374085 (HS67)
   - A/duck/Italy/99 (HS71)
   - A/duck/Scotland/180/HS77
   - A/duck/Shimane/124R/80 (HS71)
   - A/duck/Mongolia/119/2008 (HS79)

4) Human origin type B virus
   - A/Adachi/1/57 (H2N2)
   - A/swine/Sendai/1708/05
   - A/Adachi/1/57 (H2N2)
   - A/swine/Sendai/942/07
   - B/Sendai/1708/05
   - B/Sendai/942/07
   - B/Adachi/40

4. Cross reactivity

No cross-reactivity was found in all the viruses and bacteria listed below.

1) Viruses
   - Adenovirus Type 1-6, 11
   - Parainfluenza virus Type 1-4
   - Rhinovirus Type 2
   - Echovirus Type 4, 6, 9, 11, 14, 16
   - Human Metapneumovirus

2) Bacteria
   - Acinetobacter baumannii
   - Bacillus cereus
   - Bacteroides fragilis
   - Branhamella catarrhalis
   - Citrobacter freundii
   - Enterococcus faecalis
   - Fusobacterium nucleatum
   - Haemophilus influenzae
   - Kingella kingae
   - Lactobacillus casei
   - Mycobacterium avium
   - Mycobacterium tuberculosis
   - Nocardia asteroides
   - Peptostreptococcus anaerobius
   - Prevotella intermedius
   - Salmonella choleraesuis (sub. minnesota)
   - Staphylococcus aureus
   - Streptococcus bovis (E Group D)
   - Streptococcus mutans
   - Streptococcus pneumoniae

INTERFERING SUBSTANCES

The following substances were found to have no effect on the results at the concentrations indicated.

Whole blood (0.2%), acetylsalicylic acid (20 mg/mL), ambroxol hydrochloride (375 ng/mL), dequalinium hydrochloride (6.25 ng/mL), oxymetazoline hydrochloride (100 ng/mL), dried Platycodon extract (555 mg/mL), disodium cromoglycate (5 mg/mL), oxymetazoline hydrochloride (200 mg/mL), calfleme (2.5 mg/mL), dexamethasone monohydrate (10 mg/mL), Naphazoline nitrate (125 mg/mL), (R)-(-)-phenylephrine hydrochloride (1 mg/mL), fluticasone propionate (127.5 mg/mL), chlorpheniramine maleate (5 mg/mL)

REFERENCES


GLOSSARY OF SYMBOLO

- CE mark of conformity
- In vitro diagnostic medical device
- Authorized representative in the European Community
- Do not reuse
- Manufacturer/Manufactured by
- Consult instructions for use
- Keep away from sunlight
- Fragile, handle with care
- Open here

C92