



# Clearview<sup>®</sup> Exact Influenza A & B

## Intended Use

For the qualitative detection and differentiation of influenza A and influenza B virus antigen directly from nasal swabs. The test is used to obtain a visual result to aid in the diagnosis of influenza A and / or influenza B infection. For professional *in vitro* diagnostic use only.

## Introduction

Influenza (commonly known as 'flu') is a highly contagious, acute viral infection of the respiratory tract. It is a communicable disease that is easily transmitted through the coughing and sneezing of aerosolized droplets containing live virus. Influenza outbreaks occur each year during the autumn and winter months. Type A viruses are typically more prevalent than type B viruses and are associated with most serious influenza epidemics, while type B infections are usually milder.

The gold standard of laboratory diagnosis is 14-day cell culture with one of a variety of cell lines that can support the growth of influenza virus.<sup>1</sup> Cell culture has limited clinical utility, as results are obtained too late in the clinical course for effective patient intervention. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) is a newer method that is generally more sensitive than culture with improved detection rates over culture of 2-23%.<sup>2</sup> However, RT-PCR is expensive, complex and must be performed in specialised laboratories.

The **Clearview Exact Influenza A & B** test provides rapid and accurate detection of both influenza A and B virus antigen and differentiates the two viruses, allowing appropriate administration of anti-viral agents.

## Test Principle

The **Clearview Exact Influenza A & B** test is an immunochromatographic membrane assay that utilizes sandwich immunoassay technology for the detection of influenza A and B viral antigens. The test consists of a dipstick device containing a membrane strip that has separate regions with immobilized influenza A and B specific monoclonal antibodies and a coloured gold conjugate that also consists of specific influenza A and B antibodies.

The swab specimens require a sample preparation step, in which the sample is eluted off the swab into an extraction reagent (R1). The device is placed in the extraction solution.

Test results are interpreted at 15 minutes based on the presence or absence of red/pink coloured lines in the influenza A and/or B test regions. A red/pink coloured Control line appears in the Control region within 15 minutes for a valid assay.

## Kit Contents and Storage

- **20/5 Pouched Devices:** Each sealed foil pouch contains 1 influenza A & B test device, 1 extraction tube containing mucolytic agent and 1 desiccant packet
- **1 x 8ml/4ml R1: Swab Extraction Reagent** (buffered salt solution with detergent)
- **20/5 Nasal Foam Swabs**
- **1 Cardboard Workstation**
- **1 Influenza A Positive / Influenza B Negative Control Swab:** inactivated influenza A virus dried onto swab
- **1 Influenza B Positive / Influenza A Negative Control Swab:** inactivated influenza B virus dried onto swab
- **Package Insert and Procedure Card**

The test kit can be stored either refrigerated or at room temperature 20°-30°C (68°-86°F) for the duration of the shelf life. Do not use the test beyond the expiry date.

## Materials Not Supplied

- Clock, timer or stopwatch

## Precautions

- Do not mix reagents from different lots.
- Do not use after stated expiry date on the kit box.
- Do not reuse device.
- Do not use devices that have become wet or if the packaging has become damaged.
- Use only the swabs that are included in the kit.
- Standard guidelines for handling infectious agents and chemical reagents should be observed throughout all procedures. All contaminated waste such as swabs, devices and extracts should be disposed of in accordance with your biohazard waste disposal protocol.
- Package insert instructions must be followed to obtain accurate results.
- Hazard information for the components under applicable European Community (EC) Directives is as follows:
  - Swab Extraction Reagent (R1) - Harmful: Contains sodium azide

R22 Harmful if swallowed. S60 This material and its container must be deposited of as hazardous waste.

- Extraction Tube - The Extraction tube contains Tris(2-Carboxyethyl) - Phosphine HCl which is classified as Corrosive R34 Causes burns. S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. (Note: The extraction tube is exempt from labelling requirements as it is in a form, which does not present a hazard when used as instructed)
- Test Strip - Safety data sheet available for professional user on request.
- All specimen-contaminated materials should be disposed of in accordance with your biohazard waste disposal protocol.

## Specimen Collection and Storage

- Insert this 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 turbinates (less than one inch in the nostril).
- Rotate the swab three times against the nasal wall. It is recommended that swab specimens be processed as soon as possible after collection. If swabs are not processed immediately they should be placed into a dry, sterile, and tightly sealed plastic tube for storage. Swabs can be stored dry at room temperature for up to 48 hours.

## Test Procedure

- If refrigerated, allow the pouch to reach room temperature 20°-30°C (68°-86°F) before opening to avoid moisture condensation on the membrane. Patient samples and controls should also be allowed to reach room temperature prior to testing.
- Review SPECIMEN COLLECTION instructions.
- Do not open the foil pouch until ready to test.
  1. Open pouch and remove device.
  2. Place the Extraction Tube in the Workstation. Hold the swab extraction reagent bottle (R1) upside down vertically. Squeeze the bottle and let the solution drop into the extraction tube freely without touching the edge of the tube. Add 6 drops of R1 to the Extraction Tube.
  3. Place the swab specimen in the Extraction Tube. Rotate the swab for approximately 10 seconds while pressing the head against the inside of the tube to release the antigen in the swab.
  4. Remove the swab while squeezing the swab head against the inside of the Extraction Tube as you remove it to expel as much liquid as

possible from the swab. Discard the swab in accordance with your biohazard waste disposal protocol.

5. Insert the device into the Extraction Tube with the arrows pointing down and start the timer.

6. Read results in 15 minutes. A strong positive result may be visible before 15 minutes. However, to confirm a negative result the complete reaction time of 15 minutes is required. Do not read results after 20 minutes.

### Interpretation of Results

The colour intensities of the lines may vary. All lines, regardless of intensity, should be interpreted as lines. Line colour and intensity may vary from sample to sample.

The influenza A line, B line and Control line regions are indicated by 'A', 'B' and 'C' respectively, located on the reverse side of the strip. *Please refer to the illustration.*

**Positive Influenza A: Two red/pink coloured lines appear** – one in the 'A' region and one in the Control region 'C'.

**Positive Influenza B: Two red/pink coloured lines appear** – one in the 'B' region and one in the Control region 'C'.

**Positive Influenza A and Influenza B: Three red/pink coloured lines appear** – one in the 'A' region, one in the 'B' region and one in the Control region 'C'.

**Negative: Only one red/pink coloured line appears** – in the Control region 'C'. No apparent red/pink coloured line is visible in the influenza A or influenza B regions.

**Invalid:** No red/pink coloured line appears in the Control region 'C' even if a line is visible in the 'A' and / or 'B' regions. The absence of the Control line is an indication of procedural error or possible reagent deterioration. A new test should be performed.

### Quality Control

The **Clearview Exact Influenza A & B** test has a procedural control included in the test. The appearance of a line in the Control region 'C' assures the correct test procedure was followed, indicating sufficient volume of fluid was used and that capillary flow occurred. The test is invalid if the control line does not appear within 15 minutes.

Good laboratory practice recommends the use of control materials. Users should follow the appropriate national and local guidelines, accrediting groups or laboratory standard Quality Control procedures concerning the running of external quality controls.

### Swab Controls

The control swabs contain inactivated dried virus and should be treated like a patient swab sample. Follow the Test Procedure from step 1. Discard these swabs in accordance with your biohazard waste disposal protocol.

Results should be interpreted as described in 'Interpretation of Test Results'.

If the controls do not perform as expected, do not interpret the test results. Repeat the test or contact your local supplier.

### Limitations of the Test

- The accuracy of the test depends on the quality of the swab sample. False negatives may result from improper sample collection or storage.
- The use of over-the-counter and prescription nasal sprays at high concentrations can interfere with results, leading to either invalid or incorrect test results.
- A positive result for influenza A and/or B does not preclude an underlying co-infection with another pathogen, therefore the possibility of an underlying bacterial infection should be considered.
- As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test but should only be made by a physician after all clinical and laboratory findings have been evaluated.

### Expected Values

Seasonal outbreaks of influenza occur worldwide in both the northern and southern hemispheres causing widespread illness each winter. It has been estimated that influenza or its complications led to an average of 107,000 deaths per winter in the European Union between 1989 and 1998<sup>3</sup> and approximately 36,000 deaths in the US each year.<sup>4</sup> An estimated 10-20% of US residents get influenza each year, 114,000 of which are hospitalised for influenza-related complications. The number of positive results found in influenza testing is dependent on many factors including the method of specimen collection, the test method used, geographic location, and the disease prevalence in specific localities.

### Performance Characteristics

#### Sensitivity

**Clearview Exact Influenza A & B** was tested with the following influenza A and influenza B strains. A discernible line at the appropriate test line was observed at the concentrations listed.

**Table 1. Clearview Exact Influenza A & B sensitivity**

Influenza A	Detection limit CEID <sub>50</sub> /Test
A/NWS/33 10 (H1N1)	2.054 x 10 <sup>3</sup>
A/Hong Kong/8/68 (H3N2)	6.22 x 10 <sup>1</sup>
A2/Aichi/2/68 (H3N2)	8.43 x 10 <sup>2</sup>
A/Port Chalmers/1/73 (H3N2)	3.16 x 10 <sup>2</sup>
A/WS/33 (H1N1)	4.45 x 10 <sup>3</sup>
A/New Jersey/8/76 (HswN1)	2.67 x 10 <sup>2</sup>
A/Mal/302/54 (H1N1)	8.43 x 10 <sup>3</sup>

Influenza B	
Brigit	1.90 x 10 <sup>5</sup> CELD <sub>50</sub>
B/R5	1.5 EID <sub>50</sub>
B/Russia/69	3.56 x 10 <sup>2</sup>
B/Hong Kong/5/72	3.56 x 10 <sup>1</sup>
B/Lee/40	3.16 x 10 <sup>3</sup>

CEID<sub>50</sub> / EID<sub>50</sub> = Chicken Embryo Infectious Dose, concentration expected to infect half of the animals exposed.

CELD<sub>50</sub> = Chicken Embryo Lethal Dose, concentration expected to kill half of the animals exposed.

### Specificity

**Clearview Exact Influenza A & B** was tested with the following viral, bacterial and fungal strains. No discernible line at either of the test-line regions was observed at the concentrations listed.

**Table 2. Specificity testing with various viral strains**

Description	Test level
Human adenovirus C	5.62 x 10 <sup>3</sup> TCID <sub>50</sub> /ml
Human adenovirus B	1.58 x 10 <sup>4</sup> TCID <sub>50</sub> /ml
Adenovirus type 10	3.16 x 10 <sup>3</sup> TCID <sub>50</sub> /ml
Adenovirus type 18	1.58 x 10 <sup>4</sup> TCID <sub>50</sub> /ml

Human coronavirus OC43	2.45 x 10 <sup>6</sup> LD <sub>50</sub> /ml
Coxsackievirus A9	2.65 x 10 <sup>4</sup> LD <sub>50</sub> /ml 1.58 x 10 <sup>5</sup> TCID <sub>50</sub> /ml
Coxsackievirus B5	1.58 x 10 <sup>7</sup> TCID <sub>50</sub> /ml
Human herpesvirus 5	1.58 x 10 <sup>4</sup> TCID <sub>50</sub> /ml
Echovirus 2	3.16 x 10 <sup>5</sup> TCID <sub>50</sub> /ml
Echovirus 3	1 x 10 <sup>4</sup> TCID <sub>50</sub> /ml
Echovirus 6	3.16 x 10 <sup>6</sup> TCID <sub>50</sub> /ml
Herpes simplex virus 1	1.58 x 10 <sup>6</sup> TCID <sub>50</sub> /ml
Human herpesvirus 2	2.81 x 10 <sup>5</sup> TCID <sub>50</sub> /ml
Human Rhinovirus 2	2.81 x 10 <sup>4</sup> TCID <sub>50</sub> /ml
Human Rhinovirus 14	1.58 x 10 <sup>6</sup> TCID <sub>50</sub> /ml
Human Rhinovirus 16	8.89 x 10 <sup>6</sup> TCID <sub>50</sub> /ml
Measles	1.58 x 10 <sup>4</sup> TCID <sub>50</sub> /ml
Mumps	1.58 x 10 <sup>4</sup> TCID <sub>50</sub> /ml
Sendai virus	8.89 x 10 <sup>7</sup> TCID <sub>50</sub> /ml
Parainfluenza virus 2	1.58 x 10 <sup>7</sup> TCID <sub>50</sub> /ml
Parainfluenza virus 3	1.58 x 10 <sup>8</sup> TCID <sub>50</sub> /ml
Respiratory syncytial virus	8.89 x 10 <sup>4</sup> TCID <sub>50</sub> /ml
Human respiratory syncytial virus (Long )	1.58 x 10 <sup>5</sup> TCID <sub>50</sub> /ml
Rubella	2.81 x 10 <sup>5</sup> TCID <sub>50</sub> /ml
Varicella-Zoster	1.58 x 10 <sup>3</sup> TCID <sub>50</sub> /ml

TCID<sub>50</sub> = Tissue Culture Infectious Dose is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated.

LD<sub>50</sub> = Lethal Dose is the dilution of virus that under the conditions of the assay can be expected to kill 50% of the suckling mice inoculated.

**Table 3. Specificity testing with the following bacterial and fungal strains**

Strain	Organisms/ml
<i>Arcanobacterium haemolyticum</i>	1 x 10 <sup>8</sup>
<i>Candida albicans</i>	1 x 10 <sup>8</sup>

<i>Corynebacterium diphtheriae</i>	1 x 10 <sup>8</sup>
<i>Enterococcus faecalis</i>	1 x 10 <sup>8</sup>
<i>Enterococcus faecium</i>	1 x 10 <sup>8</sup>
<i>Escherichia coli</i>	1 x 10 <sup>8</sup>
<i>Haemophilus parahaemolyticus</i>	1 x 10 <sup>8</sup>
<i>Moraxella catarrhalis</i>	1 x 10 <sup>8</sup>
<i>Neisseria gonorrhoeae</i>	1 x 10 <sup>8</sup>
<i>Neisseria lactamica</i>	1 x 10 <sup>8</sup>
<i>Neisseria meningitidis</i> serogroup A	1 x 10 <sup>8</sup>
<i>Neisseria sicca</i>	1 x 10 <sup>8</sup>
<i>Neisseria subflava</i>	1 x 10 <sup>8</sup>
<i>Pseudomonas aeruginosa</i>	1 x 10 <sup>8</sup>
<i>Proteus vulgaris</i>	1 x 10 <sup>8</sup>
<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	1 x 10 <sup>8</sup>
<i>Staphylococcus epidermidis</i>	1 x 10 <sup>8</sup>
<i>Staphylococcus saprophyticus</i>	1 x 10 <sup>8</sup>
<i>Streptococcus agalactiae</i>	1 x 10 <sup>8</sup>
<i>Streptococcus bovis</i>	1 x 10 <sup>8</sup>
<i>Streptococcus dysgalactiae</i> subsp. <i>dysgalactiae</i>	1 x 10 <sup>8</sup>
<i>Streptococcus oralis</i> formerly <i>Streptococcus mitis</i>	1 x 10 <sup>8</sup>
<i>Streptococcus pneumoniae</i>	1 x 10 <sup>8</sup>
<i>Streptococcus salivarius</i>	1 x 10 <sup>8</sup>
<i>Streptococcus pyogenes</i>	1 x 10 <sup>8</sup>
<i>Streptococcus sp.</i> group F, type 2	1 x 10 <sup>8</sup>

Negative results were observed for all the viral, bacterial and fungal strains tested indicating that **Clearview Exact Influenza A & B** is specific for influenza A and influenza B.

#### Accuracy

A study of **Clearview Exact Influenza A & B** and reverse transcriptase polymerase chain reaction (RT-PCR) was carried out in a multi-centre evaluation. Nasal swabs were collected from adult and child patients exhibiting influenza-like symptoms. One swab was used to perform the

**Clearview Exact Influenza A & B** test and one swab was used for RT-PCR analysis.

The results for influenza A are summarized in Table 4 and the results for influenza B are summarized in Table 5. Clinical Sensitivity, Specificity and Overall Accuracy for **Clearview Exact Influenza A & B** are calculated based on this data.

**Table 4. Influenza A: Clearview Exact Influenza A & B correlation with RT-PCR**

	Clearview +	Clearview -	
RT-PCR +	85	19	104
RT-PCR -	2	129	131
	87	148	235

Sensitivity = (85 / 104) = **81.7%**

Specificity = (129 / 131) = **98.5%**

Overall agreement = (214 / 235) = **91.1%**

**Table 5. Influenza B: Clearview Exact Influenza A & B correlation with RT-PCR**

	Clearview +	Clearview -	
RT-PCR +	39	5	44
RT-PCR -	5	186	191
	44	191	235

Sensitivity = (39 / 44) = **88.6%**

Specificity = (186 / 191) = **97.4%**

Overall agreement = (225 / 235) = **95.7%**

#### Advice Line

Further information can be obtained from your distributor, or call Inverness Medical Technical Support on:

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