QuickVue Influenza METHOD AND SAMPLE COLLECTION

1. PURPOSE AND SCOPE

The purpose and scope of this document is to describe in some detail the procedure used by healthcare professionals for identifying influenza antigens A and B in nasal or naso-pharyngeal swabs from symptomatic residents.

2. HAZARDS

Patient Samples
All patient samples should be treated as potentially infectious and handled appropriately. Standard precautions should be employed. Personal Protective Equipment (e.g. gloves and safety glasses) should be worn when processing all samples and quality control testing.

3. CLINICAL SIGNIFICANCE

Influenza, otherwise known as the flu, is a viral respiratory infection that spreads from person to person through coughing, sneezing, and contact with contaminated surfaces. It is caused by virus types A and B, or more rarely C. influenza A The most common cause is influenza A sometimes leading to flu pandemics and epidemics. During each flu season, generally all year round in Australia, multiple strains of influenza are present, with one or two strains predominating in any particualr year.

Influenza A and B viruses undergo a a series of genetic changes over time so that people no longer have immunity from prior infections or vaccinations. An influenza epidemic is when large numbers of people are susceptible to the virus. In the case of influenza A it can undergo a major genetic change to make the virus strain much more lethal and sometimes easier to transmit. Every year experts including those in the World Health Organization design flu vaccines based on which strains are likely to circulate in the community and usually contain attenuated or inactivated virus targeting two influenza A strains and one influenza B strain.

The influenza virus causes illness in humans and in many animals, including birds, pigs (swine), dogs, and horses. Human influenza strains pass easily from person to person, but most strains of animal influenza only rarely infect humans. When they do, it is almost exclusively when there is significant close animal contact, such as a person that raises chickens or pigs, and the subsequent infection is only rarely transmitted from the infected person to another person.

Various types of flu testing are avilable, generally using nasal or naso-pharyngeal swabs, including rapid testing which can be used to guide treatment and the more accuate molecular based testing.

4. TEST PRINCIPLE

The influenza antigens are detected in clinical samples using the technique of lateral flow immunoassay. Within the test strip are highly sensitive monoclonal antibodies that react specificically with the influenza antigens and do not react with normal flora or other known respiratory pathogens.

The test requires an initial extraction of influenza A and B viral antigens. This is achieved by placine the patient sample into the reagent tube, where the virus particles are disrupted and this exposes the viral nucleoproteins. Folloing extraction, the test strip is placed in the reagent tube where the nucleoproteins react with the monoclonal antibodies in the test strip.

If the extracted specimen contains influenza A or B antigens, a pink-to-red test line along with a blue procedural Control Line will appear on the Test Strip indicating a positive result. The Test Line for influenza A or B will develop at separate specified locations on the same Test Strip. If influenza A or B antigens are not present, or are present at very low levels, only the blue procedural Control Line will appear.

5. LIMITATIONS AND KNOWN INTERFERENCE

- Whole blood, and several over-the-counter (OTC) products and common chemicals were evaluated and no significant interfence was observed with the QuickVue Influenza A+B Test at the levels tested:

Whole blood (2%); three OTC mouthwashes (25%); three OTC throat drops (25%); three OTC nasal sprays (10%); 4-Acetamidophenol (10 mg/mL); Acetylsalicylic Acid (20 mg/mL); Chlorpheniramine (5 mg/mL); Dextromethorphan (10 mg/mL); Diphenhydramine (5 mg/mL); Ephedrine (20 mg/mL); Guaiacol glyceryl ether (20 mg/mL); Oxymetazoline (10 mg/mL); Phenylephrine (100 mg/mL); and Phenylpropanolamine (20 mg/mL).

- Individuals who received nasally administered influenza A vaccine may have positive test results for up to 3 days after vaccination.

- The test is not intended to detect Influenza C antigens.

- Negative results do not preclude influenza virus infection and should not be the sole basis for treatment.

- Children tend to shed virus more abundantly and for longer periods of time than adults. Therefore, testing specimens from adults will often yield lower sensitivity than testing specimens from children.

- The QuickVue Influenza A+B Test was evaluated with a total of 62 bacterial and viral isolates. Bacterial isolates were evaluated at a concentration between 107 and 109 org/mL. Viral isolates were evaluated at a concentration of at least 104–108 TCID50/mL Adenovirus 18 and Parainfluenza virus 3 were tested at 102 TCID50/mL. None of the organisms or viruses listed below in the table below gave a positive result in the QuickVue Influenza A+B Test.

Analytical Specificity and Cross-Reactivity Bacterial Panel:	Viral Panel:
Acinetobacter calcoaceticus	Adenovirus 5 (Ad. 75)
Bacteroides fragilis	Adenovirus 7 (Gomen)
Bordetella pertussis	Adenovirus 10 (J.J.)
Branhamella catarrhalis	Adenovirus 18 (D.C.)
Candida albicans	Coronavirus OC43
Corynebacterium diphtheriae	Coxsackievirus A9 (Bozek)
Enterococcus faecalis	Coxsackievirus B5 (Faulkner)
Escherichia coli	Cytomegalovirus (Towne)
Gardnerella vaginalis	Echovirus 2 (Cornelis)
Haemophilus influenzae	Echovirus 3 (Morrisey)
Klebsiella pneumoniae	Echovirus 6 (D'Amori)
Lactobacillus casei	Herpes simplex virus 1
Lactobacillus plantarum	Herpes simplex virus 2
Legionella pneumophila	Human Rhinovirus 2 (HGP)
Listeria monocytogenes	Human Rhinovirus 14 (1059)
Mycobacterium avium	Human Rhinovirus 16 (11757)
Mycobacterium intracellulare	Measles (Edmonston)
Mycobacterium tuberculosis	Mumps (Enders)
Mycoplasma orale	Parainfluenza virus 1 (Sendai)
Mycoplasma pneumoniae	Parainfluenza virus 2 (CA/Greer)
Neisseria gonorrhoeae	Parainfluenza virus 3 (C243)
Neisseria meningitidis	Respiratory Syncytial virus (A-2)
Neisseria sicca	Respiratory Syncytial virus
Neisseria subflava	(Subgroup A, Long chain)
Proteus vulgaris	Rubella (RA 27/3)
Pseudomonas aeruginosa	Varicella-Zoster (Ellen)
Serratia marcescens	
Staphylococcus aureus	
Staphylococcus epidermidis	
Streptococcus mutans	
Streptococcus pneumoniae	
Streptococcus pyogenes	
Streptococcus sanguis	
Streptococcus sp. Gp. B	
Streptococcus sp. Gp. C	
Streptococcus sp. Gp. F	
Streptococcus sp. Gp. G	

6. PERFORMANCE CHARACTERISTICS

Clinical evaluation

The performance of the QuickVue Influenza A+B Test was evaluated in a prospective multi-center field clinical study during two distinct influenza seasons in the United States, from February to May 2017 and October 2017 to January 2018. In this clinical study, the performance of the QuickVue Influenza A+B Test was compared to an FDA-cleared influenza A and B molecular assay. The study was conducted at five (5) clinical sites, which were CLIA waived test environments comprised of Urgent Care, Pediatric and General Practice offices. A total of forty-eight (48) operators from the five sites participated in the study. In the multi-center, point-of-care (POC) field trial, two (2) nasal or two (2) nasopharyngeal swab specimens were collected from a total of one thousand one hundred and eighty-three (1183) patients. All clinical samples were collected from symptomatic patients meeting the inclusion and exclusion criteria. Thirty-nine percent (39%) of the patients tested were <5 years of age, forty-one percent (41%) 5-<18 years of age, and twenty percent (20%) ≥18 years of age. Forty-eight percent (48%) were male and fifty-two percent (52%) were female. On-site testing of one nasal swab or nasopharyngeal swab specimen in the QuickVue Influenza A+B Test was performed by CLIA waived test operators within one (1) hour of collection. This swab was incubated for one (1) minute with the extraction reagent solution before addition of the dipstick. The other swab was placed in viral transport media and stored at 2°C to 8°C prior to testing with the FDA-cleared influenza A and B molecular assay.

Analytical Sensitivity

Limit of detection was determined using a total of forty-eight (48) strains of human influenza viruses: thirty-five (35) influenza A and thirteen (13) influenza B .

Viral Strain	Viral Type	Sub-Type	Minimum Detectable Level	Viral Strain	Viral Type	Sub-Type	Minimum Detectable Level
			$TCID_{50}$/mL				**pfu/mL****
New Caledonia/20/99	A	H1N1	1.63×10^{3}	Fort Monmouth/1/47	A	H1N1	6.70×10^{3}
California/04/09*	A	H1N1	4.4×10^{3}	Aichi	A	H3N2	3.20×10^{3}
				Shangdong	A	H3N2	8.40×10^{3}
			EID_{50}/mL	Maryland/91	A	H1N1	1.00×10^{4}
A/Anhui/1/2013*	A	H7N9	7.90×10^{6}	Japan/305/57	A	H2N2	1.30×10^{4}
				Johannesburg/94	A	H3N2	1.44×10^{4}
			pfu/mL**	Brazil	A	H1N1	1.70×10^{4}
Hong Kong	A	H3N2	6.60×10^{-1}	Sydney	A	H3N2	2.00×10^{4}
Beijing/32/92	A	H3N2	3.30×10^{0}	Bangkok	A	H3N2	3.30×10^{4}
Shanghai/11	A	H3N2	6.70×10^{0}	Wuhan	A	H3N2	3.30×10^{4}
Shanghai/16	A	H3N2	1.00×10^{1}	Beijing/353/89	A	H3N2	3.30×10^{5}
Victoria	A	H3N2	3.30×10^{1}	Singapore/86	A	H1N1	6.60×10^{5}
Singapore/1/57	A	H2N2	6.70×10^{1}	Texas/91	A	H1N1	1.60×10^{7}
Port Chalmers	A	H3N2	1.24×10^{2}	Victoria	B		1.40×10^{4}
USSR	A	H1N1	2.00×10^{2}	Taiwan	B		1.10×10^{2}
Puerto Rico/8/34	A	H1N1	2.60×10^{2}	Panama	B		1.00×10^{0}
New Jersey	A	H1N1	2.70×10^{2}	Ann Arbor	B		3.30×10^{2}
Taiwan	A	H1N1	3.30×10^{2}	Singapore	B		3.30×10^{2}
Tokyo/3/67	A	H2N2	3.40×10^{2}	Lee	B		6.60×10^{2}
Bayern	A	H1N1	6.60×10^{2}	Hong Kong	B		7.00×10^{2}
Sichuan	A	H3N2	6.60×10^{2}	Beijing/184/93	B		1.66×10^{3}
Beijing/352/89	A	H3N2	7.70×10^{2}	California	B		3.30×10^{3}
NWS/33	A	H1N1	1.00×10^{3}	Maryland	B		6.60×10^{3}
Fort Warren/1/50	A	H1N1	1.70×10^{3}	Yamagata/16/88	B		6.70×10^{3}
Mississippi	A	H3N2	1.70×10^{3}	Harbin	B		1.40×10^{4}
Texas/77	A	H1N1	3.30×10^{3}	Stockholm	B		3.30×10^{5}

$TCID_{50}$/mL = 50% tissue culture infectious dose ; EID_{50}/mL = 50% egg infective dose; pfu/mL = plaque-forming unit per milliliter.

*Although this test has been shown to detect the 2009 H1N1 and H7N9 viruses cultured from a positive human respiratory specimens, the performance characteristics of this device with clinical specimens that are positive for the 2009 H1N1 or H7N9 infuenza viruses have not been established. The QuickVue Influenza A+B Test can distinguish between influenza A and B viruses, but it cannot differentiate influenza subtypes.

**These viral strains were obtained from American Type Culture Collection (ATCC) with titer information, and the titers were not verified by Quidel. The performance characteristics for influenza A virus subtypes emerging as human pathogens have not been established.

Analytical reactivity was further evaluated using a total of twenty-four (24) influenza A viruses isolated from birds and mammals. The QuickVue Influenza A+B Test detected all of the strains examined

Viral Strain*	Viral Type	Viral Subtype
Duck/Tottori/723/80	A	H1N1
Duck/Alberta	A	H1N1
Duck/Hokkaido/17/01	A	H2N2
Duck/Mongolia/4/03	A	H3N8
Duck/Ukraine/1/63	A	H3N8
Equine/Miami/1/63	A	H3N8
Duck/Czech/56	A	H4N6
Hong Kong/483/97	A	H5N1
Hong Kong/156/97	A	H5N1
Chicken/Yamaguchi/7/04	A	H5N1
A/Chicken/Vietnam/33/04	A	H5N1
A/Vietnam/3028/04	A	H5N1
A/Thailand/MK2/04	A	H5N1
Duck/Pennsylvania/10128/84	A	H5N2
Turkey/Massachusetts/3740/65	A	H6N2
Seal/Massachusetts/1/80	A	H7N7
Turkey/Ontario/67	A	H8N4
Turkey/Wisconsin/66	A	H9N2
Chicken/Germany/N/49	A	H10N7
Duck/England/56	A	H11N6
Duck/Alberta/60/76	A	H12N5
Gull/Maryland/704/77	A	H13N6
Mallard/Astrakhan/263/82	A	H14N5
Duck/Australia/341/83	A	H15N8

PRECISION STUDIES

The total, within-run, and between-run performance of the QuickVue Influenza A+B Test was evaluated for precision. A panel consisting of two different levels of influenza A antigen (Johannesburg/82/96; weak positive and strong positive) and two different levels of influenza B antigen (Harbin/7/94; weak positive and strong positive) were repeated five times with a single lot of QuickVue Influenza A+B Test on three different days. One hundred percent (100%) accuracy was obtained for all specimens tested.

7. STORAGE STABILITY

Store the kit at room temperature (2-30^{o}C), out of direct sunlight. Do not freeze the kit. Kit materials are stable until the expiration date printed on the outer box. It is recommended to perform the test immediately after removing the test device from the foil pouch as it is sensitive to humidity and temperature.

8. SPECIMEN REQUIREMENTS

Nasal Swab Sample:

For optimal test performance with a Nasal Swab specimen, use the Swabs supplied in the kit.
It is important to obtain as much secretion as possible. Therefore, to collect a nasal swab sample, 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 turbinates (less than one inch into the nostril). Rotate the Swab a few times against the nasal wall.

Nasopharyngeal Swab Sample:

It is important to obtain as much secretion as possible. Therefore, to collect a nasopharyngeal swab sample, carefully insert the sterile Swab into the nostril that presents the most secretions under visual inspection. Keep the Swab near the septum floor of the nose while gently pushing the Swab into the posterior nasopharynx. Rotate the Swab several times.

Specimens should be tested immediately after collection.

9. QUALITY CONTROL

Built-in quality control

The Test Strip contains a built-in procedural control feature with a two-colour result format providing a simple interpretation for positive and negative results.

The appearance of a blue procedural Control Line provides several forms of positive control by demonstrating sufficient flow has occurred and the functional integrity of the Test Strip was maintained. If the blue procedural Control Line does not develop at 10 minutes, the test result is considered invalid and should be repeated with another test strip

A built-in negative control is provided by the clearing of red background colour, verifying that the test has been performed correctly. Within 10 minutes, the result area should be white to light pink and allow the clear interpretation of the test result. If background colour appears and interferes with interpretation of the test result, the result is considered invalid. Should this occur, review the procedure and repeat the test with a new Test Strip.

External Quality Control

External controls are also available in the kit to demonstrate that the reagents and assay procedure perform properly.

These positive and negative controls should be run as part of the training of an operator and for each new lot number of test strips. The controls are tested using the same procedure as for Nasal/Nasopharyngeal Swab samples.

If the controls do not perform as expected, repeat the test or contact APPN before testing patient specimens.

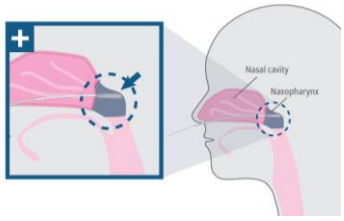
All control results should be recorded in the Results database and marked as a Quality Control Sample.

10. TEST PROCEDURE

Sample Collection

1. Wash your hands and apply the necessary Personal Protective Equipment

2. Use a sterile swab supplied with the test kit to collect a naso-pharyngeal sample from the nostril of the patient, reaching the surface of the posterior nasopharynx.



3. Using gentle rotation, push the swab until resistance is met at the level of turbinate.

4. Rotate the swab several times, against the nasopharyngeal wall.

5. Withdraw the sterile swab from the nasal cavity.

Specimen testing

1. Dispense all of the Reagent Solution into the Reagent Tube. Gently swirl the tube to dissolve its contents.

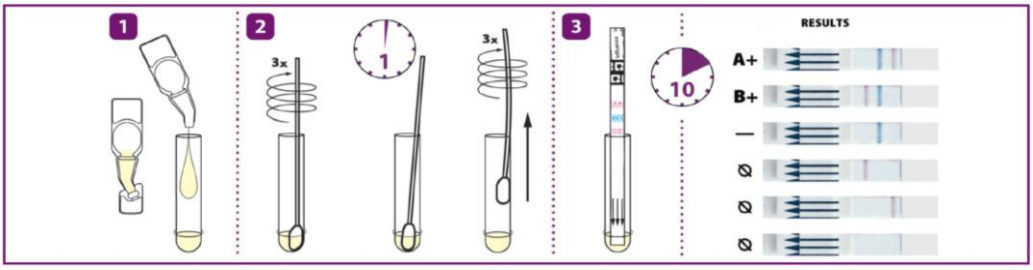
2. Place the patient swab with sample into the reagent tube. Roll the swab at least 3 times while pressing the head against the bottom and side of the reagent tube.

Leave the swab in the reagent tube for 1 minute.

3. Roll the swab head against the inside of the reagent tube as you remove it. Dispose of the used swab in accordance with your biohazard waste disposal protocol.

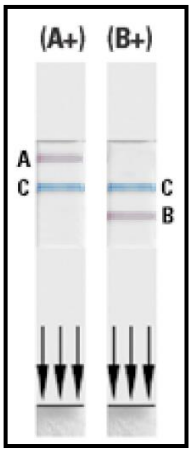
4. Place the test strip into the reagent **tube** with the arrows on the test strip pointing down. Do not handle or move the Test Strip until the test is complete and ready for reading.

5. Read result at 10 minutes. Some positive results may appear sooner. Do not read result after 10 minutes.



Result interpretation

Positive Result:



At 10 minutes, the appearance of ANY shade of a pink-to-red Test Line, either above or below the blue Control Line, AND the appearance of a blue procedural Control Line indicates a positive result for the presence of influenza A and/or B viral antigen.

- If the red line is above the Control Line, the test results are positive for type A. See image to the immediate right (A+).
- If the red line is below the Control Line, the test results are positive for type B. See image to the far right (B+).

Negative Result:

At 10 minutes, the appearance of ONLY the blue procedural Control Line indicates influenza A and B viral antigen were not detected.

A negative result should be reported as a presumptive negative for the presence of influenza antigen.

Invalid Result:

If at 10 minutes, the blue procedural Control Line does not appear, even if any shade of a pink-to-red Test Line appears, the result is considered invalid.

If at 10 minutes, the background colour does not clear and it interferes with the reading of the test, the result is considered invalid.

If the test is invalid, a new test should be performed with a new patient sample and a new Test Strip.

11. RESULTS

Enter the result together with the subject details (Name, Unit No), test date and test time, into the APPN database.

12. REFERENCES

This method has been adapted from the QuickVue Influenza A+B Test (2020).