ARBOR VITA CORPORATION

A/H7N9 Influenza Rapid Test

For Use Under an Emergency Use Authorization (EUA) Only

Instructions for Use

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Intended Use

The A/H7N9 Influenza Rapid Test is intended in conjunction with clinical and epidemiological information:

- For the in vitro qualitative detection of NS1 protein antigen from influenza A/H7N9 virus (detected in China in 2013) directly from symptomatic patient nasal swab specimens, and viral culture. The test may react with NS1 protein antigen of other Asian avian Influenza viruses such as, A/H9N2, A/H5N1, and A/H10N8.

- For the presumptive identification of viral infection in patients who may be infected with influenza A/H7N9 virus (detected in China in 2013) from nasal swabs, and viral culture in conjunction with clinical and epidemiological risk factors. The test may react with other Asian avian Influenza viruses such as, A/H9N2, A/H5N1, and A/H10N8.

- To provide epidemiologic information for surveillance of influenza A/H7N9 virus (detected in China in 2013).

Testing with the A/H7N9 Influenza Rapid Test should only be used in conjunction with other laboratory testing and clinical observations for the presumptive identification of patients infected with influenza A/H7N9 virus (detected in China in 2013).

A negative test is presumptive and it is recommended that these results be confirmed by an FDA-cleared influenza A and B molecular assay. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to USA state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

This test should be used by Department of Defense (DoD) network laboratories in the U.S. and outside the U.S. or other U.S. government laboratories outside the U.S. for testing U.S. citizens living and traveling abroad in China and other affected areas, and for U.S. military, Department of State, and other U.S. governmental agency personnel stationed and working in China and other affected areas who may potentially be exposed to influenza A/H7N9 virus (detected in China in 2013) or be exposed to individuals who may carry the influenza A/H7N9 virus (detected in China in 2013), or by foreign laboratories.

The AVC A/H7N9 Influenza Rapid Test is for use under the Food and Drug Administration's Emergency Use Authorization only.

Summary and Explanation

Since the first report of the A/H7N9 virus from China in March 2013, there have been two waves of infection resulting in 411 reported cases and 124 fatalities as of April 4, 2014. The second wave 2013/ 2014 proved bigger with 275 cases reported to date compared to the first wave from 2013 at 136 cases[1-12]. All the infections appeared to originate from China and spread through human migration to Hong Kong, Taiwan and Malaysia despite vigilant border scrutiny. The CDC has concluded that it is possible for human cases of A/H7N9 flu to be found in the United States with the most likely scenario through a traveler from China[1]. The CDC has issued a health alert. This test should be used for U.S. citizens living and traveling abroad in China and other affected areas, and for U.S. military, Department of State, and other U.S. governmental agency personnel stationed and working in China and other affected areas who may potentially be exposed to A/H7N9 (detected in China in 2013) or be exposed to individuals who may carry the influenza A/H7N9 virus (detected in China in 2013). As a "novel" (non-human) virus it has the potential to cause a pandemic if it were to become easily and sustainably spread from person-to-person. Although this virus has not shown that ability to date, influenza viruses constantly change and it is possible that this virus could gain that ability with a few mutations or as few as one mutation[2].

Explanation

To meet the pandemic preparedness needs of USA civilian and military personnel abroad and in the USA, the US Navy and AVC collaborated to develop a simple field test to identify influenza A/H7N9 virus (detected in China in 2013) using a lateral flow immunoassay that will allow field personnel with minimal training to perform and requiring no cold chain. The resultant test detects NS1 protein, encoded by one of the six core genes reported to be shared between all influenza A strains from birds that have infected humans (H7N9, H9N2, H10N8 and H5N1) as reported in Lancet[12]. The NS1 protein is expressed in the cell within 24 hours following infection and may aid in the early diagnosis of illness caused by avian influenza in patients with ILI (influenza like illness).

Test Procedure

The A/H7N9 Influenza Rapid Test detects the presence of influenza A/H7N9 from nasal swabs collected from patients with ILI. A clinical specimen is transferred to an A/H7N9 Influenza Rapid Test Lysis Tube, containing A/H7N9 Influenza Rapid Test Lysis Buffer, where cells are lysed releasing intracellular proteins (including NS1). The resultant lysate is added to the sample well of the A/H7N9 Influenza Rapid Test Cassette. The NS1 from the specimen reacts with detector monoclonal antibodies and antibodies on the membrane of the cassette. Each Test Cassette produces a visual read out consisting of three different indicator lines. The indicator lines are for influenza A/H7N9

virus (detected in China in 2013) **(Line 2)**, Pan influenza A control including seasonal Influenza A and A/H7N9 viruses **(Line 1)**, and an internal positive control **(C)** (Figure 1). A presumptive positive result will require all three test lines to be visible (Figure 2). The A/H7N9 Influenza Rapid Test Positive Control is included to demonstrate qualitative functionality of the test.

Warnings and Precautions

- **Biohazard.** Biological samples such as tissues, body fluids, and blood have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations.
- Use routine laboratory precautions. Do not eat, drink, or smoke in designated work areas.
- Wear suitable protective equipment when using this test. Use of Nitrile or Latex gloves is recommended when handling patient samples.
- To obtain accurate results, you must follow the Instructions for Use. Do not use kit components beyond the expiration date.
- If test components or chemicals appear damaged or irregular, do not use them.
- If the internal positive control line is not visible on the cassette, do not use the test result.
- Inadequate or inappropriate specimen collection, storage, and transport may yield erroneous test results.
- Specific training or guidance is recommended if operators are not experienced with specimen collection and handling procedures.

Important Public Health and Surveillance Information

Centers for Disease Control and Prevention (CDC) recommends maintaining the enhanced surveillance efforts by state and local health departments, hospitals, and clinicians to identify patients at increased risk for A/H7N9 avian influenza. Guidelines for enhanced surveillance are as follows:

1. Patients with new-onset of severe acute respiratory illness requiring hospitalization (i.e., illness of suspected infectious etiology that is severe enough to require inpatient medical care in the judgment of the treating clinician). **AND**
2. History of travel within 10 days of symptom onset to a country with documented A/H7N9 avian influenza in poultry and/or humans.

Testing for avian influenza A/H7N9 should be considered on a case-by-case basis in consultation with state and local health departments for hospitalized or ambulatory patients with:

1. Documented temperature of >38°C (>100.4°F), **AND**
2. One or more of the following symptoms: cough, sore throat, shortness of breath, **AND**

3. History of contact with poultry (e.g., visited a poultry farm, a household raising poultry, or a bird market) or a known or suspected human case of influenza A (A/H7N9) in an A/H7N9-affected country within 10 days of symptom onset.

The above recommendations are subject to change; please refer to current recommendations posted on the CDC website:
http://www.cdc.gov/flu/avianflu/h7n9/testing.htm

Specimens

A/H7N9 Influenza Rapid Test is for use with nasal swab specimens, and viral cultures.

Specimen Collection and Handling

Proper specimen collection and handling is critical to the performance of this test. Specimens should be tested as soon as possible after specimen collection.

Two swabs should be taken from suspect case. One swab is for use in rapid testing and the other swab is placed in VTM and sent to a USA public health laboratory for more accurate influenza testing. USA public health authorities should be notified of any suspected institutional outbreak and respiratory specimen should be collected from ill persons (whether positive or negative by RIDT).

Note: *For proper test performance, samples should be tested immediately after sample collection. Adequate swabs include Diagnostic Hybrid's nasal swab FLOQSwab™ (Cat. #502CS01, and #503CS01).*

Nasal Swab Sample
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 several times against the nasal wall until sufficient material is collected.

Kit Storage and Stability

Store the A/H7N9 Influenza Rapid Test kit at 36–86°F (2–30°C), away from direct sunlight. Kit contents are stable prior to the expiration date printed on the outer box. Do not freeze any of the kit components.

Materials Required (Provided)

Part number #1200000

Table 1. Kit Contents (10 tests per Kit)

A/H7N9 Influenza Rapid Test Lysis Buffer (Part #1200300)	1 bottle
A/H7N9 Influenza Rapid Test Cassette (Part #1200100)	10 cassettes
A/H7N9 Influenza Rapid Test Positive Control (Part #1200500)	1 tube
A/H7N9 Influenza Rapid Test Lysis Tubes* (Part #1200400)	10 tubes
Instructions for Use	1 each
Quick Guide	1 each

*Each tube contains dried reagents

Materials Required (But Not Provided)

Sterile Flocked Swabs

Equipment and Consumables Required (But Not Provided)

Micropipette and micropipette tips for use with samples in viral transport media (M4 or UTM).

Quality Controls

Built In Control Features

Each A/H7N9 Influenza Rapid Test Kit contains a built-in procedural control feature. When running the test, the appearance of a red Control Line in each test indicates proper functioning of the buffer reagents, capillary flow, and functional integrity of the test strip within the cassette. If the Control Line does not appear, the test is considered Invalid. A second control line is included in the cassette, and is labeled Line 1. The control Line 1 contains monoclonal antibody(ies) that detect NS1 protein from all influenza A strains (Pan-influenza A). If influenza A is present in the sample (seasonal Flu A or A/H7N9), Line 1 should turn red. Line1 must be present for a diagnosis of influenza virus A/H7N9 (detected in China in 2013).

External Quality Control

Each A/H7N9 Influenza Rapid Test Kit contains an external Positive Control. The positive control contains recombinant influenza A/H7N9 NS1 protein in dried form (non-pathogenic), that must be re-suspended before use (see Test Procedure). Lysis Buffer (requires preparation, see Test Procedure) should be used as the Negative Control. External positive and negative controls should be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. At a minimum, a positive and a negative control test must be performed and the expected results documented prior to using each kit for the first time. The kit should not be used if external control tests do not produce the correct results. Repeat the external control tests as the first step in determining the root cause of the failure. Contact Arbor Vita Corporation Customer Support (408-585-3939) for assistance when control failures are repeated. The kit should be used for patient specimens only if both the positive and negative controls tests give expected results.

Controls included in the Kit

a. Internal positive control **(C)** (built into each test cassette)
b. A/H7N9 Influenza Rapid Test Positive Control which contains recombinant influenza A/H7N9 NS1 protein in dried form (non-pathogenic), that must be re-suspended before use (see Test Procedure). One Positive control is included in each Kit.

Procedure

Prior to beginning the test, ensure that all clinical specimens and test materials are at room temperature. Check the expiration on each individual reagent and outer kit box before using the test. Do not use any tests past the expiration date on the label. Use Universal Biological Precautions when handling any clinical specimen.

A. Sample Preparation

1. Check the expiration for each individual reagent and outer kit box before using the test. Do not use any tests past the expiration date on the label.
2. Use recommended swabs to collect nasal specimen.

Figure 1. Test Workflow

B. Running the Test

1. Remove one Lysis Tube from pouch.
2. Remove A/H7N9 Influenza Rapid Test Lysis Buffer from kit. Using dropper from Lysis Buffer bottle cap (in vertical position), add **14 drops** of Lysis Buffer to an opened Lysis Tube.
3. Close Lysis Tube and make sure yellow cap remains on tube's nozzle. Mix contents by gently inverting tube 10 times. Gently tap bottom of tube on solid surface to collect solution towards bottom of tube.
4. Perform nasal sample lysis

   Place clinical specimen swab into Lysis Tube containing Lysis Buffer and mix by swirling the tip of the swab against the wall of the Lysis Tube for at least **10 seconds**. Properly dispose of clinical specimen swab.
5. Close the lid of the Lysis Tube and remove yellow cap from nozzle of tube. The tube can now be used as a dropper.
6. Remove A/H7N9 Influenza Rapid Test Cassette from pouch and place face-up on a flat horizontal surface. Note: Apply sample to cassettes immediately after opening the cassette pouch.
7. Transfer **4 drops** of sample from Lysis Tube to the Test Cassette sample well (S) at ~45^0 angle.
8. Read result at **20 minutes**.

C. Interpretation of Results and Reporting

1. Test is **NEGATIVE for Influenza A/H7N9 virus (detected in China in 2013)** if:
   A. Only control line (C) is visible OR
   B. The control line (C) and Pan influenza A (Line 1) are visible but not Line 2

   A negative result is a presumptive negative and should be followed by an FDA-cleared influenza device with subtyping capabilities for all currently circulating influenza A viruses in the United States (i.e., seasonal A/H3, and A/H1 pandemic). If

clinically indicated, the patient can be retested or patient sample be sent for further testing using molecular assays.

2. Test is **INCONCLUSIVE for Influenza A/H7N9 virus (detected in China in 2013)** if: control line (C) and A/H7N9 (Line 2) are present.

   Contact U.S. public health laboratories, or DoD network laboratories immediately for coordination of additional testing and for further guidance.

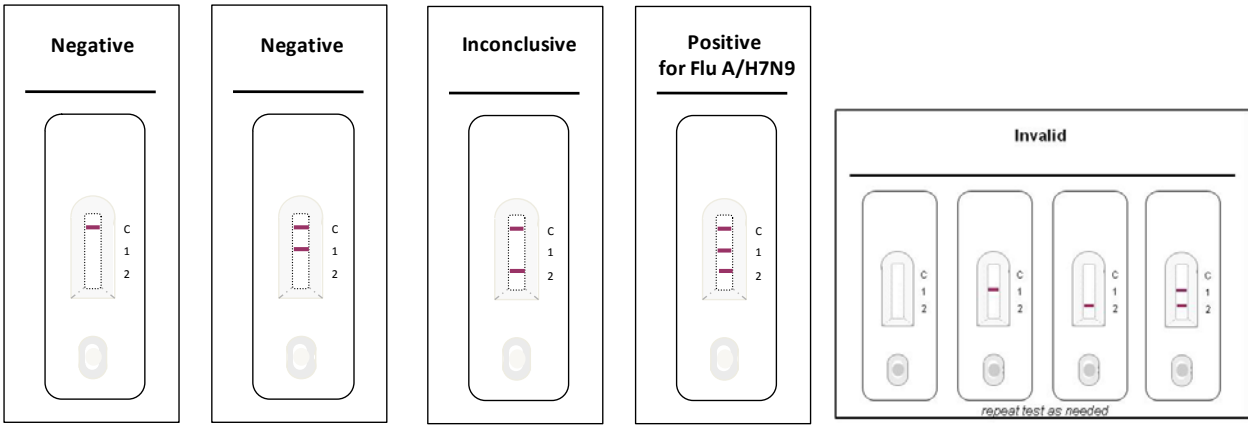
3. Test is **POSITIVE for Influenza A/H7N9 virus (detected in China in 2013)** if: control line (C), Pan influenza A (Line 1) and A/H7N9 Test (Line 2) are visible.

   IMMEDIATELY report specimen presumptive positive for influenza A/H7N9 virus (detected in China in 2013) to the U.S. public health authorities, or DoD* network laboratories *to coordinate transfer of the specimen for additional molecular testing.

4. Test is **INVALID** if: No control line (C) is seen.

   An INVALID result should be repeated. If clinically indicated, the patient sample may be sent for further studies.

Figure 2. Test Interpretation



D. Running A/H7N9 Influenza Rapid Test Positive Control

1. Remove A/H7N9 Influenza Rapid Test Positive Control pouch from kit box. Remove A/H7N9 Influenza Rapid Test Positive Control tube and yellow cap from pouch. Remove yellow cap from tube and set aside for use in step #3.

2. Using dropper from Lysis Buffer bottle cap (in vertical position), add **14 drops** of Lysis Buffer to Positive Control tube.
3. Close the lid of the lysis tube. Place the yellow cap over the tube nozzle.
4. After **5-10 minutes**, mix components by gentle shaking of capped tube.
5. Transfer **4 drops** of sample from Positive Control tube to the Test Cassette sample well (S) at ~45^0 angle.
6. Read result at **20 minutes**. All three Test lines (C, #1 and #2) should be visible.

E. Running A/H7N9 Influenza Rapid Test Negative Control

1. Remove one Lysis Tube from pouch.
2. Remove A/H7N9 Influenza Rapid Test Lysis Buffer from kit. Using dropper from Lysis Buffer bottle cap (in vertical position), add **14 drops** of Lysis Buffer to an opened Lysis Tube.
3. Close Lysis Tube and make sure yellow cap remains on tube's nozzle. Mix contents by gently inverting tube 10 times. Gently tap bottom of tube on solid surface to collect solution towards bottom of tube.
4. Remove yellow cap from nozzle of tube. The tube can now be used as a dropper.
5. Remove A/H7N9 Influenza Rapid Test Cassette from pouch and place face-up on a flat horizontal surface. Note: Apply Lysis Buffer to cassettes immediately after opening the cassette pouch.
6. Transfer **4 drops** of Lysis Buffer from Lysis Tube to the Test Cassette sample well (S) at ~45^0 angle.
7. Read result at **20 minutes**. The Control Line (C) should be visible and Line1 should not be visible.

The definitive identification of influenza A/H7N9 virus (detected in China in 2013), either directly from patient specimens or from viral cultures, requires additional laboratory testing along with clinical and epidemiological assessment in consultation with national influenza surveillance experts.

Limitations of the Procedure

- For *emergency* use only.
- Although this test has been shown to detect cultured human-derived influenza A subtype A/H7N9 virus (detected in China in 2013), the performance characteristics of this test with direct specimens from humans infected with A/H7N9 or other avian influenza viruses are unknown.
- Results obtained with this test must be confirmed and be used in conjunction with other laboratory testing and clinical and epidemiological assessments in consultation with the clinician evaluating the patient and influenza surveillance experts.

- In analytical testing, closely related Influenza A/H9N2 avian isolates, and 1997/98 A/H5N1 were detected as A/H7N9-positive. This test may react with newly identified A/H10N8 infecting humans based on bioinformatics analysis.
- Some historical (not current) seasonal influenza A strains may lead to a false A/H7N9- positive test interpretation at high viral titers. Examples of such influenza A strains include A/PR8/34 (H1N1) and A1/Denver/1/57 (H1N1).
- Failure to follow the Test Procedure and Instructions on Interpretations of Test Results may adversely affect test performance and/or invalidate the Test Result.
- The detection of the NS1 protein of A/H7N9 influenza virus (detected in China in 2013) is dependent upon proper specimen collection, handling, storage, and preparation, including lysis. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
- If the level of antigen in a sample is below the detection limit of the test, a negative test result may occur.
- Negative test results do not exclude the presence of other influenza or non-influenza viral infections and should not be used as the sole basis for treatment or other patient management decisions.
- Positive test results do not exclude co-infections with other viral or bacterial pathogens.
- Monoclonal antibodies may fail to detect, or detect with less sensitivity, influenza A/H7N9 viruses that have undergone amino acid changes in the target epitope region.

Performance Characteristics

Clinical Performance

Retrospective Study Results

The clinical specificity of the A/H7N9 Influenza Rapid Test Flu was evaluated at NHRC with retrospective clinical samples derived from nasal swabs from five categories of patients: negative for influenza A/B, adenovirus and rhinovirus; positive for influenza A/H3N2 only; positive for A/H1N1 (pandemic) only; positive for influenza B only; positive for rhinovirus only. All samples were collected during the 2013-2014 influenza season. The study included both pediatric and adult populations. Symptomatic subjects with a history of fever and (sore throat, rhinorrhea, or cough) were recruited from four clinic sites into a respiratory illness surveillance study conducted by the Naval Health Research Center (NHRC). The samples were collected into 2.5 ml of Universal Transport Media (UTM). Testing was performed according to the Instructions for Use of the A/H7N9 Influenza Rapid Test. The study included testing of a total of 57 symptomatic subjects in a blinded fashion. Of these patients, **20** had influenza A, **10** had influenza B and **27** were influenza A/B-negative. The performance of the A/H7N9 Influenza Rapid Test is summarized in **Table 2**. All samples tested negative for influenza A/H7N9 virus (detected in China in 2013) giving 100% negative percent agreement with the expected result.

Table 2. Performance Summary of the A/H7N9 Influenza Rapid Test Using Retrospective Samples

	Actual Condition of Population			Performance
AVC Test Result w/ Nasal Samples	**Patients with A/H7N9**	**Patients without A/H7N9**	**Totals**	
A/H7N9-Positive	0 (true positives)	0 (false positives)	0	N/A
A/H7N9-Negative	0 (false negatives)	57 (true negatives)	57	NPA: 100% (CI 93.7% - 100 %)
Invalid	0	0	0	
Totals	0	57	57	

Performance characteristics using contrived specimen

Study I: Spiking with recombinant A/H7N9 NS1 protein

Due to the lack of available clinical specimens from patients with A/H7N9 virus, evaluation of the performance of A/H7N9 Rapid Test was carried out using an alternative approach. The surrogate A/H7N9-positive clinical samples were generated by spiking *recombinant* A/H7N9 NS1 protein (A/Anhui/1/2013_H7N9) at various concentrations into individual nasal swab lysate from asymptomatic patients. The NS1 concentrations included the negative control (no spiking) and NS1 spiked at Low (2X LoD), Moderate (4X LoD) and High (10X LoD) concentrations. These samples were tested in the study in a randomized and blinded fashion. The test performance results are summarized in **Table 3**.

Table 3. Performance Summary Using Clinical Surrogate Samples Generated by Spiking Recombinant A/H7N9 NS1 protein (A/Anhui/1/2013_H7N9) into Individual Nasal Clinical Samples

Sample type	Expected Result	Actual Result
A/H7N9-negative samples	4/4	4/4
Low A/H7N9-positive contrived samples	7/7	7/7
Moderate A/H7N9-positive contrived samples	7/7	6/7 *
High A/H7N9-positive contrived samples	6/6	6/6
PPA: 95 % (95% CI: 76.4%-99.1 %) (n=20 surrogate A/H7N9 positive samples) NPA: 100 % (95 % CI: 51.0 % -100 %) (n=4 nasal swab clinical samples)		

* The single negative, the pan flu A (line 1) was negative suggesting that the sample did not contain NS1.

Study II: Spiking with A/Anhui/2013_H7N9 virus culture

In a second study, surrogate A/H7N9-positive clinical samples were generated by spiking A/Anhui1/2013_H7N9 *viral culture* (supernatant + cells) at various concentrations into individual nasal swab lysate from asymptomatic patients. The viral culture concentrations included the negative control (none spiked) and viral culture spiked at LoD (1X LoD), Low (2X LoD), Moderate (4X LoD) and High (10X LoD) concentrations. The identity of samples was blinded to the operator. The test performance results are summarized in **Table 4**.

Table 4. Performance Summary Using Clinical Surrogate Samples Generated by Spiking A/Anhui/1/2013_H7N9 Viral Culture (Supernatant and Infected Cells) into Individual Nasal Clinical Samples

Sample type	Expected Result	Actual Result
A/H7N9-negative samples	4/4	4/4
A/H7N9-positive contrived sample at 1X LoD	7/7	7/7
Low A/H7N9-positive contrived samples	7/7	7/7
Moderate A/H7N9-positive contrived samples	7/7	7/7
High A/H7N9-positive contrived samples	6/6	6/6
PPA: 100 % (95 % CI: 87.5% -100%) (n=27 surrogate A/H7N9 positive samples) NPA: 100 % (95 % CI: 51.0 % -100 %) (n=4 nasal swab clinical samples)		

Analytical Performance

A. Limit of Detection (LoD)

Using Recombinant NS1 protein detection

The Limit of detection (LoD) of the A/H7N9 Influenza Rapid Test was determined using recombinant NS1 protein (A/Anhui/1/2013_H7N9 strain). The LoD was defined as the lowest detectable level of NS1 protein at which 95% of the replicates tested positive. The Pan Flu A control (line 1) detects 10 pg of A/H7N9 NS1 protein and the A/H7N9 test line (line 2) detects 250 pg of A/H7N9 NS1 protein.

Table 5. LoD Determination Study Results (Recombinant NS1 protein)

Virus Strain	Analytical LOD, NS1 protein		Characteristics
	Pan Flu A	A/H7N9	
A/Anhui/1/2013_H7N9	10 pg per test*	250 pg per test*	NS1 Recombinant protein
**Test is 100 µl of specimen lysate*			

Using Viral Culture

The sensitivity of the A/H7N9 Influenza Rapid Test was determined using an A/Anhui/1/2013_H7N9 virus. The reported limit of detection (LoD) is based on measurements taken from influenza A/Anhui/1/2013_H7N9 viral culture samples containing culture supernatant and infected cells and confirmed with replicates (n=23). The NS1 protein target is an intracellular marker of infection and therefore infected cells also contribute to the test signal.

Table 6. LoD Determination Study Results (Viral Culture)

Virus Strain	LoD $TCID_{50}$/mL	
A/Anhui/1/2013_H7N9	Pan Flu A	A/H7N9
	$3.2x10^3$	$3.2x10^5$

B. Analytical Reactivity (Inclusivity) and Specificity (Exclusivity)

The analytical reactivity (inclusivity) and specificity (exclusivity) performance of the A/H7N9 Influenza Rapid Test was evaluated at Arbor Vita Corporation, NHRC (Naval Health Research Center, San Diego, CA), NRL (Naval Research Laboratory, Washington DC), and Hong Kong University. The following strains were tested using viral cultures or recombinant proteins. The A/H7N9 Influenza Rapid Test detects human A/H7N9 and does not detect seasonal Influenza A as well as pandemic A/H1N1. The Pan Flu A control was positive for all influenza A strains evaluated.

Analytical Reactivity (Inclusivity)

Table 7. Analytical Reactivity Study Results (A/H7N9 strains)

Virus Strain	Reactivity		Characteristics
	Pan Flu A	A/H7N9	
A/Shanghai/1/2013_H7N9 **,*	++++	++++	Human A/H7N9
A/Shanghai/2/2013_H7N9 **,*	++++	++++	Human A/H7N9
A/Anhui/1/2013_H7N9 **,*	++++	++++	Human A/H7N9
A/turkey/Minnesota/1/1988_H7N9**	++++	+	Pre-2013 bird H7N9
** tested on viral culture ** tested on recombinant protein*			

The performance of the A/H7N9 Influenza Rapid Test was also evaluated using influenza strains with NS1 sequences closely related to A/H7N9 human isolates. It is known that 6 core genes (not HA and N) from influenza A/H9N2 were acquired by current influenza A/H7N9, A/H10N8 and A/H5N1. The A/H7N9 Influenza Rapid Test detected A/H9N2 isolates from chickens, the newly identified A/H10N8 infecting humans based on informatics analysis and the

1997/98 A/H5N1. After 1998, the A/H5N1 viruses are quite different and are not recognized by the A/H7N9 Influenza Rapid Test.

Table 8. Analytical Reactivity Study Results (non-A/H7N9 strains)

Virus Strain	Reactivity		Characteristics
	Pan Flu A	A/H7N9	
A/Hong Kong/97/98_H5N1**	++++	++++	Early Human A/H5N1
A/chicken/Hong Kong/TC8/2012(H9N2)*	++++	+++	Chicken A/H9N2 NS1 is highly similar to Human A/H7N9 NS1
A/chicken/Hong Kong/ FY64W/2010(H9N2)*	++++	+++	
A/chicken/Hong Kong/ SSP176/2009(H9N2)*	++++	+	

** tested on viral culture ** tested on recombinant protein*

A/H7N9 Influenza Rapid Test also is predicted to detect the newly identified Influenza A/Jiangxi-Donghu/346/2013 (H10N8) virus based on the fact that A/H7N9 and A/H10N8 viruses share the same hypothetical Antibody-recognition epitope.

Analytical Specificity (Exclusivity)

The Pan-Flu A control (line 1) reacted with all influenza A viral culture strains tested. All influenza A strains (listed in Tables 9 and 10 in this section) tested negative for A/H7N9 (line 2) at the indicated viral titers. However, two historical (not current) influenza A strains (A/PR8/34 (H1N1) and A1/Denver/1/57 (H1N1)) tested A/H7N9-positive at high titer levels (≥2.2x10^7 $CEID_{50}$/ml).

Table 9. Analytical Specificity Study Results (Non-A/H7 Influenza A Viruses)

Viruses	ATCC Code	Final Titer, $TCID_{50}$/mL (T) or $CEID_{50}$/mL (C)	Titer Source	Test Result
A2/Wisconsin/67/2005 (H3N2-like)	VR-544	2.22E+08 C	ATCC	Negative
A/Hiroshima/52/2005 (H3N2-like)	VR—547	3.95E+07 C	ATCC	Negative
A/Port Chalmers/1/73 (H3N2)	VR-810	3.95E+07 C	ATCC	Negative
A/Victoria/361/2011 (H3N2)	NA	1.41E+06 T	NHRC	Negative
A/Perth/16/2009 (H3N2)	NA	1.69E+05 T	NHRC	Negative
A/Mexico/4108/2009 (H1N1)	NA	7.91E+05 T	NHRC	Negative
A/California/07-2009 (H1N1)	NA	1.69E+04 T	NHRC	Negative
A1/Denver/1/57 (H1N1)	VR-546	2.22E+07 C	ATCC	Positive*

A/PR8/34 (H1N1)	VR-95	2.22E+07 C	ATCC	Positive*
B/Hong Kong/5/72	VR-823	2.22E+06 C	ATCC	Negative

* A/H7N9-negative test result was obtained for A/PR8/34 (H1N1) and A1/Denver/1/57 (H1N1) at 10-fold lower viral titer or 2.22E+06.

Table 10. Analytical Specificity Results (Non-A/H7 Influenza A recombinant NS1 protein)

Virus Strain	Reactivity		Characteristics
	Pan Flu A	A/H7N9	
A/California/04/2009_H1N1	+++	-	2009 Pandemic human H1N1
A/Brevig Mission/1/1918_H1N1	++	-	1918 Pandemic human H1N1
A/Taiwan/112/1996_H1N1	++++	-	Seasonal human H1N1
A/New York/31/2004_H3N2	+++	-	Seasonal human H3N2
A/Vietnam/1194/2004_H5N1	++++	-	Human A/H5N1

C. Analytical Specificity/Cross-Reactivity-Non-Influenza Bacterial and Respiratory Viral Pathogens

The A/H7N9 Influenza Rapid Test was evaluated for potential cross-reactivity with a total of 40 bacterial and viral isolates. The bacterial isolates were tested at concentrations of approximately $7.5x10^7$ cfu/mL. The viral isolates were used at concentrations of $7x10^3 – 10^9$ $TCID_{50}$/mL. Testing for each organism was performed in duplicate. None of the tested pathogens listed below showed cross-reactivity with the assay; samples reported A/H7N9-negative.

Table 11. Cross-Reactivity Study Results-Bacteria

Pathogen	ATCC Code	Final Titer, cfu/mL	Test Results
Bacteroides fragilis	ATCC 23745	7.5x10(7)	Negative
Bordetella pertussis	ATCC 8467	7.5x10(7)	Negative
*Corynebacterium sp. (*C. xerosis)	ATCC 373	7.5x10(7)	Negative
Escherichia coli	ATCC 43888	7.5x10(7)	Negative
Haemophilus influenza	ATCC9006	7.5x10(7)	Negative
Lactobacillus sp. (Lactobacillus casei)	ATCC 334	7.5x10(7)	Negative
Legionella pneumonphila	ATCC 33153	7.5x10(7)	Negative
Moraxella catarrhalis	ATCC 25238	7.5x10(7)	Negative
Neisseria meningitides	ATCC 13077	7.5x10(7)	Negative

Neisseria mucosa	ATCC 19694	7.5x10(7)	Negative
Peptostreptococcus anaerobius	ATCC 27337	7.5x10(7)	Negative
Porphyromonas asaccharolyticus	ATCC 25260	7.5x10(7)	Negative
Pseudomonas aeruginosa	ATCC 10145	7.5x10(7)	Negative
Staphylococcus aureus	ATCC 11632	7.5x10(7)	Negative
Staphylococcus epidermidis	ATCC 12228	7.5x10(7)	Negative
Streptococcus pneumonia	ATCC 10015	7.5x10(7)	Negative
Streptococcus pyogenes Group A	ATCC 49399	7.5x10(7)	Negative
Streptococcus salivarius	ATCC 7073	7.5x10(7)	Negative
Streptococcus sp. Group B	ATCC 27956	7.5x10(7)	Negative
Streptococcus sp. Group C	ATCC 9528	7.5x10(7)	Negative

Table 12: Cross-Reactivity Study Results-Viruses

Pathogen	ATCC Code	Final Titer, $TCID_{50}$/mL	Test Results
Adenovirus, Type 2	VR-846	3.95E+06	Negative
Adenovirus, Type 3	VR-3	3.95E+08	Negative
Adenovirus, Type 7	VR-7	2.22E+05	Negative
Adenovirus, Type 14	VR-15	3.95E+08	Negative
Coronavirus OC43	VR-1558	3.95E+05	Negative
Coronavirus 299E	VR-740	3.95E+04	Negative
Coxsackievirus Type A9	VR-1311	2.22E+06	Negative
Coxsackievirus Type B5	VR-185	7.03E+07	Negative
Cytomegalovirus	VR538	7.03E+03	Negative
Echovirus Type 3	VR-33	7.03E+05	Negative
Echovirus Type 6	VR-36	3.95E+05	Negative
Enterovirus	VR-283	3.95E+06	Negative
HSV Type 1	VR-260	7.03E+07	Negative
Measles virus	VR-24	3.95E+05	Negative
Parainfluenza Type 1	VR-94	7.03E+03	Negative
Parainfluenza Type 2	VR-92	2.22E+06	Negative
Parainfluenza Type 3	VR-93	3.95E+07	Negative
Rhinovirus Type 1A	VR-1559	3.95E+06	Negative
Respiratory Syncytial virus Type A	VR-1540	2.22E+06	Negative
Respiratory Syncytial virus Type B	VR-1400	7.03E+04	Negative

Troubleshooting

Problem	Potential Cause	Mitigation
Control line (C) is not visible after running test	1) Sample incorrectly added to Test Cassette 2) Test components stored incorrectly 3) Test Cassette defective	1) Obtain new test components and the sample and rerun 2) Obtain new test components and sample and rerun 3) Check for visible damage and contact manufacturer
All three Test lines (C, #1, and #2) not visible after running Positive Control	1) Positive Control not incubated for 5-10 minutes or properly mixed 2) Positive Control sample not properly added to Test Cassette	1) Mix remaining Positive Control sample in tube and rerun using new Test Cassette 2) Rerun Positive Control by correctly adding 4 drops of sample to the new Test Cassette

Problem	Potential Cause	Mitigation
Drops of sample can't be squeezed out of Lysis Tube	1) Tip of Lysis Tube cap is plugged 2) Lysis Tube is cracked	1) Transfer 100 uL of sample to Test Cassette sample well using a pipette 2) Transfer 100 uL of sample to Test Cassette sample well using a pipette
Test Cassette membrane is pink in color	1) Backflow of sample due to extended run time (>>20 min)	1) Obtain new Test Cassette and rerun sample for 20 minutes

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A/H7N9 Influenza Rapid Test Quick Guide

Result Interpretation will change