

Test Characteristics of the Anigen® Rapid AIV Ag Test (Avian Influenza Type A Rapid Antigen Test) in Ducks in Vietnam

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Abstract

The current study evaluated the test characteristics of a commercially available rapid antigen test (The Anigen® Rapid AIV Ag test) for Highly Pathogenic Avian Influenza (HPAI). Diagnostic specimens were collected from 175 ducks at markets and under controlled laboratory conditions in Vietnam. The relative diagnostic test sensitivity and the relative diagnostic test specificity were calculated by using real-time Reverse Transcription Polymerase Chain Reaction tests as the confirmatory diagnosis. The Anigen® Rapid AIV Ag test had a relative diagnostic sensitivity of 0.61 (95% confidence interval [CI] 0.45-0.75) and a relative diagnostic specificity of 0.98 (95% CI: 0.94–0.99). The result show that the Anigen® test can detect, with limited sensitivity, HPAI H5N1 clades circulating in Vietnam. The test can aid by making a differential diagnosis in sick or dying chickens, ducklings and adult ducks. Due to the limited sensitivity more birds may need to be tested to increase flock sensitivity. As flock prevalence of virus shedding ducks in Vietnam is low, this test is not suitable to reliably and quickly detecting Influenza A virus from apparently healthy ducks.

Keywords: Antigen test; Avian influenza; H5N1; Ducks; Sensitivity; Specificity ; Vietnam

Content

Highly Pathogenic Avian Influenza (HPAI) (subtype H5N1) is endemic in poultry in Vietnam (OIE World Organization for Animal Health, "Update on highly pathogenic avian influenza in animals" <http://www.oie.int/animal-health-in-the-world/update-on-avian-influenza/2014/>). HPAI outbreaks are reported frequently and all over the country. The disease is fatal in unvaccinated chickens and ducklings. Adult ducks (*Anas platyrhynchos*) are not always affected, depending on virus clade (WHO, 2011. H5N1 viruses are grouped into numerous virus "clades" based on the phylogenetic characterization and sequence homology of the hemagglutinin (HA) gene, http://www.who.int/influenza/gisrs_laboratory/h5n1_nomenclature/en/) and the infectious doses received. Subclinical infected adult ducks silently shed the virus into the environment infecting susceptible poultry. Currently, to detect H5N1 virus in ducks, oropharyngeal diagnostic specimens are collected and sent to a regional laboratory for real-time reverse transcription polymerase chain reaction (rRT-PCR) testing. This is time consuming and once the results are known, the sampled ducks have moved on in the poultry value chain, possible spreading H5N1 virus. Avian Influenza type A rapid antigen tests have been evaluated before, but never in Vietnam on ducks. This study reports on the relative diagnostic test sensitivity and the relative diagnostic test specificity of the rapid antigen test, Anigen® Rapid AIV Ag test (hereafter Anigen test) and makes recommendations for field applications. The study design and reporting is similar to earlier work, so comparisons can be made [1].

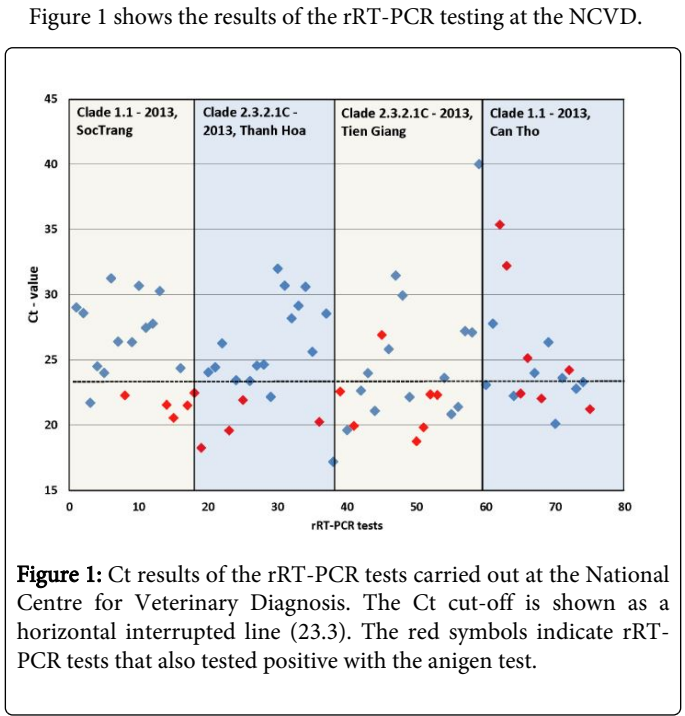
The study was conducted in 2 stages at 2 different locations. The first part of the study was conducted in 3 markets in the town of Vinh,

in the province of Nghe An, Vietnam in August 2013. Experienced laboratory staff collected diagnostic specimens from live, apparently healthy, ducks at the markets. From each of the 100 birds, 2 oropharyngeal swabs were taken. Immediately the Anigen test was conducted according to the manufacturers' instructions [According to the manufacturer's website; this test can detect all Orthomyxoviridae Avian Influenza viruses with a Hemagglutinin envelop structure (H1-H16) in birds, with a minimum detection limit of 0.125 Hemagglutination Units (HAU)]. The results were recorded. The second swab was placed in BD viral transport medium, kept at 40°C, and was delivered the same day to the Regional Animal Health Laboratory (RAHO) III. Here the specimens were held frozen at -70°C until further testing. Once all specimens were received, rRT-PCR tests with both matrix and hemagglutinin 5 (H5) gene primers were used to confirm or exclude H5 gene. Swab samples were extracted manually using Qiagen Viral RNA (Qiagen, Hilden, Germany) kits and then screened by rRT-PCR to detect the influenza virus A Matrix (M) gene. All M gene positives were subsequently tested using H5 primer/probe pairs, and all H5 positives were tested for N1. The RAHO used the same primer/probe sets designed and validated internally at the National Centre for Veterinary Diagnosis (NCVD, the nominated national reference laboratory for avian influenza in Vietnam), and were distributed together with reagents, positive controls, VTM, and sampling consumables through the national laboratory network. The threshold cycle used to determine a positive sample was Ct ≤ 23.3 for both M gene and H5 rRT-PCR assays.

The second part of the study was conducted at the NCVD in Hanoi, Vietnam in November 2013. Here, under biosecurity level 3+ conditions, 40 ducks were experimental challenged with Influenza A H5N1 clade 1.1 or clade 2.3.2.1C. On day 3, day 12 and on the day of the death of the bird (if occurred) two oropharyngeal swabs were

taken. Immediately, the Anigen test was conducted according to the manufacturers' instructions. The results were recorded. The second swab was placed in BD viral transport medium, kept at 4°C, and was held frozen at -70°C until further testing. Once all 75 specimens were received, rRT-PCR tests with both matrix and hemagglutinin 5 (H5) gene primers were used to confirm or exclude H5 gene. The exact same diagnostic protocol and primers were used as at the RAHO.

The relative diagnostic test sensitivity (Sensitivity relates to the test's ability to identify the H-gen correctly and is calculated as: the number of true positive tests divided by the number of true positive tests + the number of false negative tests) and the relative diagnostic test specificity (Specificity relates to the test's ability to exclude the H-gen correctly and is calculated as: the number of true negatives divided by the number of true negatives + the number of false positives) were calculated by using rRT-PCR as the confirmatory diagnosis or "gold standard"[2]. Confidence intervals were calculated by using the Wilson score method without continuity correction [3].



Time post-inoculation	Number tests	of	Positive test	rRT-PCR	Positive test	Anigen
Day 3	37		14		9	
Day 12	11		0		0	
Day of death	29		18		16	
Total	75		32		25	

Table 1:Results of the real-time Reverse Transcription Chain Reaction test (rRT-PCR) and the anigen test the National Centre for Veterinary Diagnosis, by time.

Table 1 shows the differences of Anigen and rRT-PCR test results at the NCVD over time. The combined number of positive and negative anigen tests and the results of the rRT-PCR test on the same group of

samples are shown in Table 2. The positive and negative results of the rRT-PCR test were used in the subsequent calculations of the Anigen field test characteristics, a total of 175 tests (Table 3).

Results	Regional Animal Health Office III		National Centre for Veterinary Diagnoses	
	Anigen	rRT-PCR	Anigen	rRT-PCR
Positive	0	4	25	32
Negative	100	96	50	43
Total	100	100	75	75

Table 2: Results of the anigen tests and the real-time Reverse Transcription Chain Reaction (rRT-PCR) tests at both locations.

rRT-PCR			
Anigen	Positive	Negative	Total
Positive	22	3	25
Negative	14	136	150
Total	36	139	175

Table 3: Two-by-two table showing the results of the anigen test compared with the rRT-PCR test.

The Anigen test had a relative diagnostic sensitivity of 0.61 (95% confidence interval [CI]: 0.45–0.75) and a relative diagnostic specificity of 0.98 (95% CI: 0.94–0.99). As PCR tests have also sensitivities and specificities less than 100%, using PCR results as gold standard may lead to classification errors. The calculated diagnostic sensitivity and specificity are estimates and are “relative”. The result show that the Anigen test can detect, with limited sensitivity, Influenza A H5N1 clade 1.1 and clade 2.3.2.1C (currently) circulating in Vietnam. It is unclear why there is such a big discrepancy between rRT-PCR and Anigen test results; the limited detection may be caused by the amount of virus shedding at time of sampling, or a reduced sensitivity for the particular Vietnam virus clades, especially related to false-negative results from the Anigen test. But compared to similar work in chickens in Indonesia the sensitivity of detected Influenza A H5 antigen in ducks is lower, this was expected [1-4].

Monitoring H5N1 in healthy ducks over the last 3 years shows that about 1% of ducks sheds virus at the time of sampling [5]. Considering the low sensitivity, the Anigen test is unsuitable to detect Influenza A H5 in healthy ducks. However, the test can aid with making a differential diagnosis when needed in sick or dying chickens, ducklings and adult ducks. Due to the limited sensitivity more birds may need to be tested to increase flock sensitivity.

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