

Peer Reviewed

Diagnosing H3N8 CIV Infection

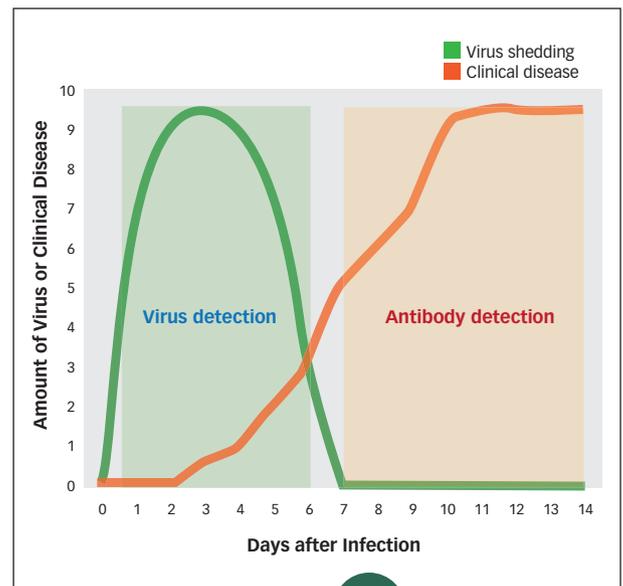
H3N8 CIV infection should be included in the differential diagnosis for all dogs with acute respiratory infection.

Canine influenza virus subtype H3N8 (H3N8 CIV) is a highly contagious respiratory pathogen. The virus causes acute onset of coughing, sneezing, and nasal discharge that persists for 2 weeks or longer in most dogs and progresses to pneumonia in some.

H3N8 CIV infection should be included in the differential diagnosis for dogs with acute respiratory infection, particularly those with a history of boarding, day care attendance, or adoption from a shelter or rescue group within a week of the onset of clinical illness.¹ CIV cannot be ruled out in H3N8 CIV-vaccinated dogs with acute respiratory disease; although currently available vaccines can reduce virus shedding and decrease the severity and duration of clinical disease, they do not protect against infection.²

Because CIV infection resembles that caused by other viral and bacterial pathogens in the canine infectious respiratory disease complex, it cannot be diagnosed by clinical signs alone.¹ CIV infection has two diagnostic windows based on the virus-shedding period and appearance of antibodies (Figure 1). Although infected dogs shed virus for about 7 days, peak virus shedding occurs during the first 2 to 4 days of infection before onset of clinical signs.³ Virus shedding declines rapidly during the first 5 days of clinical disease.³ Serum antibodies to H3N8 CIV are detectable after 7 days and increase during the first month after infection.^{3,4}

Diagnostic test selection for H3N8 CIV infection is based on the diagnostic window that applies at the time of presentation. Virus detection tests can be used for dogs that have been ill for 5 or fewer days and are still in the virus-



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Diagnostic windows for H3N8 CIV infection are based on the virus-shedding period and appearance of antibodies. Diagnostic tests for virus can be used in dogs that have been ill for 5 or fewer days (orange line) and are still in the virus-shedding window (green line). In dogs that have been ill for longer than 5 days, diagnosis relies on antibody testing, as these dogs are no longer shedding virus.

CONTINUES

CIV = canine influenza virus

shedding window. Antibody detection tests are needed for dogs that have been ill for longer than 5 days and are outside the virus-shedding window.

PCR TESTING

Polymerase chain reaction (PCR) testing detects viral nucleic acids in clinical samples using primers for the influenza A matrix gene and/or the CIV hemagglutinin (*H3*) gene.¹ PCR can detect minute amounts of viral nucleic acid and the virus does not have to be infectious. Of importance, the current H3N8 CIV vaccines containing inactivated virus do not interfere with PCR testing.

Two national reference laboratories (Antech Diagnostics and IDEXX Laboratories) offer PCR testing for a panel of canine respiratory pathogens that includes H3N8 CIV (Table 1). Cornell University Animal Health Diagnostic

Center (CU-AHDC) offers PCR testing that initially screens for the highly conserved influenza A matrix gene, followed by testing positive samples for the CIV *H3* gene.

Indications

PCR is used for virus detection in swabs or transtracheal and bronchoalveolar washes collected from dogs that have been ill for 5 or fewer days. Polyester or cotton swabs with plastic handles are preferable, but bacterial culturettes can be used. The sample collector should wear clean examination gloves for each dog to minimize sample contamination.

Nasal and deep pharyngeal swabs should be collected and pooled for each dog to maximize virus detection (Figure 2). Swabs submitted to Antech or IDEXX should be left dry. A few drops of sterile saline should be added to the tube for

swabs submitted to CU-AHDC. Samples should be stored in a refrigerator and submitted to the laboratory on cold packs, ideally on the day of collection.

Advantages

PCR is the most sensitive and rapid test for diagnosing H3N8 CIV in the acute phase of disease.¹ The turnaround time of 1 to 3 days allows timely clinical management of individual patients and formation of control strategies for kennel outbreaks. PCR testing may also identify other influenza A virus subtypes that infect dogs.

Disadvantages

The sensitivity of PCR is highly dependent on sample collection during the virus-shedding period. False-negative results can occur when samples are collected from dogs that have been ill for longer than 5 days.

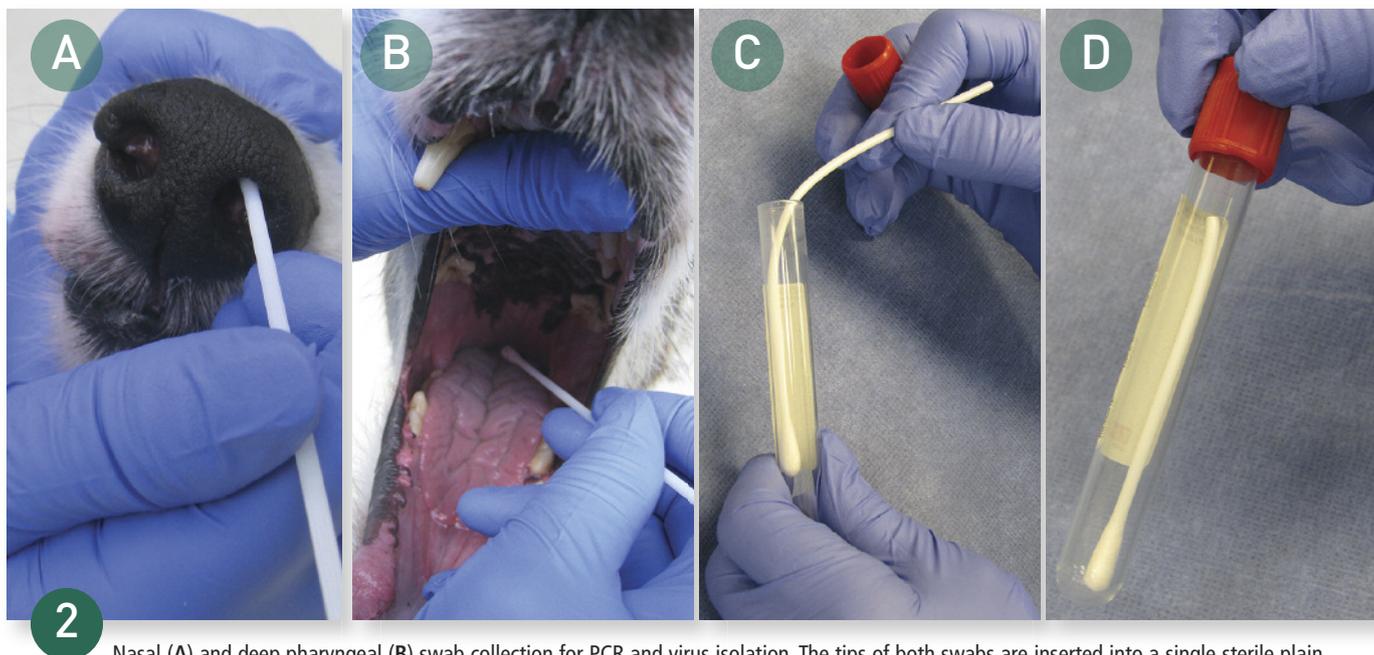
For best accuracy, negative PCR and virus isolation results should be confirmed with serology before ruling out H3N8 CIV infection.



Table 1. Antech and IDEXX Canine Respiratory Pathogen PCR Panels

FastPanel PCR Canine Respiratory Disease Profile (Antech Diagnostics)	Canine Respiratory Disease (CRD) RealPCR Panel (IDEXX Laboratories)
<i>Bordetella bronchiseptica</i>	<i>Bordetella bronchiseptica</i>
Canine respiratory coronavirus	Canine respiratory coronavirus
Canine adenovirus type 2	Canine adenovirus type 2
Canine distemper virus	Canine distemper virus
Canine parainfluenza virus	Canine parainfluenza virus
Canine herpesvirus	Canine herpesvirus type 1
Canine influenza virus (H3N8)	Canine influenza virus (H3N8)
H1N1 influenza virus	H1N1 influenza virus
H5N1 influenza virus	<i>Mycoplasma cynos</i>
<i>Mycoplasma cynos</i>	<i>Streptococcus equi</i> subsp <i>zoepidemicus</i>
<i>Streptococcus equi</i> subsp <i>zoepidemicus</i>	

CIV = canine influenza virus, CU-AHDC = Cornell University Animal Health Diagnostic Center, PCR = polymerase chain reaction



2 Nasal (A) and deep pharyngeal (B) swab collection for PCR and virus isolation. The tips of both swabs are inserted into a single sterile plain red-top or similar tube (C), and the plastic handles are snapped (D) to release the tips into the tube.

VIRUS ISOLATION

Isolation of H3N8 CIV from clinical samples confirms the diagnosis of canine influenza.

Indications

Similar to PCR testing, virus isolation is useful for diagnosing dogs that have been ill for 5 or fewer days. Swabs are collected and prepared as described for PCR testing, and lower respiratory tract washes may also be collected. Clinical samples should be submitted on cold packs to CU-AHDC.

Advantages

Virus isolation is vital for molecular and antigenic characterization of H3N8 CIV isolates to determine whether mutations that may influence the efficacy of diagnostic tests or vaccines have occurred. Virus isolation may also identify other influenza A virus subtypes that infect dogs.

Disadvantages

Virus isolation is less sensitive than PCR testing because the former depends on the presence of infectious virus in sufficient amounts for recovery in culture.¹ The turnaround time for results is much longer than for PCR testing, making virus isolation less useful in timely decision making for clinical management of individual patients and control of kennel outbreaks.

SEROLOGY

Serologic diagnosis of H3N8 CIV infection depends on testing of paired acute and convalescent serum samples for virus-specific antibodies.¹ Serum samples should be collected during the first week of illness and again 2 weeks later.

Seroconversion, defined as a fourfold rise in antibody titer between acute and convalescent samples, is diagnostic of recent active virus infection as long as the dog was not vaccinated during the testing period.² Serum samples can be submit-

ted to CU-AHDC or to Antech. Samples should be stored in a refrigerator pending submission to the laboratory.

Indications

In many cases, dogs potentially exposed to H3N8 CIV are not presented for examination until they have been ill for 5 days, making serologic diagnosis the only option. Because of the potential for false-negative results with PCR and virus isolation, testing paired acute and convalescent sera is the ideal confirmatory option.¹

Advantages

The hemagglutination inhibition assay used for H3N8 CIV antibody detection is highly sensitive and specific.^{1,4} This assay is sufficient for diagnosing CIV as long as paired sera are tested and the dog has not been vaccinated against H3N8 CIV during the test period.

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Disadvantages

The main disadvantage of serology is the long turnaround time for paired sample results. This test cannot provide a timely answer for early clinical management of individual patients or for control of kennel outbreaks.

CLOSING REMARKS

Proper diagnosis of H3N8 CIV infection is important for patient management and timely control of outbreaks in kennel facilities. Reliability and associated costs for each testing method are presented in **Table 2**. The best approach for accurate diagnosis is collection of swabs and serum from dogs within the first 5

days of clinical disease, followed by serum collection 2 weeks later.

Swabs are submitted for PCR for quick virus screening and potential virus isolation. Since negative results do not rule out infection, paired sera should be submitted for confirmatory antibody testing.

 Table 2. Reliability and Economic Impact of Available Diagnostic Tests		
Test	Reliability	Cost
PCR	<ul style="list-style-type: none"> • Depends on sample collection from dogs in early phase of infection, proper sample collection and handling, and stringent matching of PCR primers with targeted viral genes • For best accuracy, confirm negative results with serology testing of paired acute and convalescent samples before ruling out infection 	Moderate: Comparable with CBC/differential and serum chemistry panel
Virus isolation	<ul style="list-style-type: none"> • Depends on sample collection from dogs in the early phase of infection • For best accuracy, confirm negative results with serology testing of paired acute and convalescent samples before ruling out infection 	Moderate: At CU-AHDC, comparable with serum chemistry panel; however, there is no additional charge for virus isolation for PCR-positive samples
Serology (hemagglutination inhibition assay)	<ul style="list-style-type: none"> • Depends on testing of paired acute and convalescent serum samples to determine whether seroconversion has occurred • Compromised by testing of convalescent samples only, especially in dogs vaccinated against H3N8 CIV 	Moderate: Comparable with CBC/differential and serum chemistry panel

CBC = complete blood count, CU-AHDC = Cornell University Animal Health Diagnostic Center, PCR = polymerase chain reaction

See Aids & Resources, back page, for references & suggested reading.

Resources

The following reference laboratories offer PCR testing for H3N8 CIV:

Antech Diagnostics:
<http://antechdiagnostics.com>

Cornell University Animal Health Diagnostic Center:
<http://ahdc.vet.cornell.edu/test/>

IDEXX Laboratories:
<http://idexx.com>