

CPV/CDV/CAV Antibody Lateral Flow Test Kit

Chinta M. Lamichhane, BVSc & AH, PhD; Adrian Alexandru, DVM, Siba K. Samal, BVSc & AH, PhD, Diplomate ACVM

Background

Canine adenovirus type-1 (CAV-1), canine distemper virus (CDV), and canine parvovirus type-2 (CPV-2) are all highly contagious infections with a high fatality rate, especially in young puppies. Since there are no effective treatments, vaccinations are the only method of controlling the spread of these infections throughout the population [1].

Canine Adenovirus Type-1

CAV-1 is a virus that causes infectious canine hepatitis and can be spread via direct contact with contaminated saliva, urine, and feces [2]. CAV-1 is potentially fatal, most often in younger dogs [2]. CAV-2 primarily causes a mild respiratory disease and is used in vaccines to provide cross-protection against the more severe CAV-1 infection [3]. However, the vaccine only lessens the severity of the infection, rather than preventing it from being spread entirely [2].

Canine Distemper Virus

CDV is passed between canines via direct contact with contaminated fresh blood, urine, or saliva, or via contaminated food and water bowls [3]. Wild animals can also carry and spread the infection [3].

Canine Parvovirus Type-2

CPV-2 is spread between canines via oral contact with feces or feces-contaminated surfaces. The infection can remain in contaminated ground for up to 5 months, or longer in favorable conditions [4]. Although vaccines can be very helpful for preventing infection, emergence of new variants of the infection can render vaccinations ineffective [4].

Symptoms and Risk Factors

Canine Adenovirus Type-1

CAV-1 can cause fever, apathy, tenderness, abdominal pain, acute/chronic hepatitis and interstitial nephritis, vomiting, diarrhea, and blood in the stool [2]. Other symptoms may include bronchopneumonia, conjunctivitis, photophobia, and a transient corneal opacity [2].

Dogs under 2 years of age are more likely to die from a CAV-1 infection than older dogs [2].

Canine Distemper Virus

CDV typically presents as watery or pus-like discharge from the eyes, followed by fever, nasal discharge, coughing, lethargy, reduced appetite, and vomiting. Neurological symptoms are also common, such as seizures, muscle twitches, paralysis, circling behavior, head tilts, and jaw convulsions. Other symptoms include hardening of the foot pads. [3]

Puppies under four months of age and dogs that have not been vaccinated are at an elevated risk of contracting CDV [3].

Canine Parvovirus Type-2

CPV-2 typically presents as lethargy, depression, loss of appetite, high fever, vomiting, and diarrhea [5]. Because the infection can cause severe vomiting and diarrhea, an infected canine is at serious risk of rapid dehydration, causing death within 48 to 72 hours of the initial symptoms [5].

Young puppies are particularly susceptible to infection, and often vaccines can fail to provide adequate protection due to lingering immunity from the mother's milk [5]. Puppies should therefore be given a series of CPV-2 vaccines, with a dose administered to the puppy when it is within 14 to 16 weeks old [5].

Vaccine Guidelines

The World Small Animal Veterinary Association (WSAVA) [6] and the American Animal Hospital Association (AAHA) [7] state that the core vaccines for dogs are those that protect against infection by CDV, CAV and CPV-2. Guidelines for vaccination indicate that vaccination for CAV, CDV, and CPV-2 is critical to the health and safety of dogs, and that since the duration of immunity is typically on the order of years, vaccines should not be given more frequently than every three years [6].

In order to ensure the necessity of vaccination, serological tests play an important role in determining the antibody status of the animal [6].

Serological Testing

The WSAVA defines the role of serology in vaccination decision making [6]. It states that the presence of virus-specific serum antibodies correlates strongly with protection from CAV, CDV, and CPV. Determination of antibody status is especially relevant for the assessment of dogs that have an unknown vaccination history or are overdue for vaccination, for informing revaccination intervals in adult dogs, and in management of infectious disease outbreaks in shelters.

The gold standard test for CAV and CDV is serum neutralization (SN). For CPV, the gold standard is hemagglutination inhibition (HI). Testing laboratories provide an antibody titer and determine whether that titer is above the threshold that is considered protective. A positive antibody result (CPV HI titer $\geq 1:80$, CDV SN titer $\geq 1:32$ and CAV SN titer $\geq 1:16$) [9] indicates that the dog has protective immunity. Antibody testing can prevent unnecessary revaccination, mitigating risk from adverse side effects. A positive test result in a dog with a history of vaccination indicates a successful protective immunity in response to vaccination. A positive result may also occur in an unvaccinated but healthy dog, which suggests prior exposure to and recovery from the infection.

A negative antibody result (CPV HI titer $< 1:80$, CDV SN titer $< 1:32$ and CAV SN titer $< 1:16$) indicates that the dog is susceptible to infection and revaccination is recommended [6]. Previously vaccinated dogs may test negative if the dog was not re-vaccinated as antibody levels will fall over time. A negative result in a dog that has previously been vaccinated suggests that the dog is a genetic non/low-responder, or that interfering levels of MDA were present at the time of vaccination.

The SN and HI tests are traditional laboratory-based tests requiring a high level of technical skill for the correct manipulation of the cell cultures and live viruses [10]. The procedures are intricate and time-consuming and must be performed in specialized diagnostic laboratories [11].

Recently, there have been advances in the availability of quick and simple on-site serological test kits that can be used to detect the presence of protective antibodies of CAV, CDV or CPV in dogs. The commercially available enzyme-linked immunosorbent assay (ELISA) tests (including VacciCheck® (Biogal Galed Labs, Isreal) [12] and Titercheck® (Zoetis, Parsippany, NJ, USA) [13]) provide an alternative to routine revaccination at 3-year intervals. The ELISA tests do not require cell cultures and have a lower risk of contamination [2]. Indirect fluorescent antibody (IFA) tests are also used. However, these antibody tests cannot distinguish between

vaccine-induced seroconversion and that caused by infection. To confirm infection, polymerase chain reaction (PCR) [14] or direct virus isolation should be used.

Lateral Flow Test Methodology

Biotech Laboratories U.S.A. LLC offers a lateral flow test kit, the RapidSTATUS™ CAV/CDV/CPV Antibody Test Kit, which detects antibodies against each virus antigen, provides rapid on-site results intended for use in determination of immune status of dogs against these viruses.

The test kit uses nitrocellulose membrane-based test strips, one each for CAV, CDV, and CPV. A drop of serum, plasma, or anticoagulated whole blood is added to the sample well of the test device. The sample reconstitutes dried gold conjugates. Two to three drops of chase buffer are then added to force reagent flow down the strip. The sample and the reconstituted conjugates flow along the test strip.

The test results can be observed visually by the user. The presence of a test and control line are interpreted as a positive result, the presence of only a control line is interpreted as a negative result, and any test without a control line is inconclusive.

Sensitivity and Specificity

The sensitivity and specificity of the test kit are based on a 2x2 analysis of data.

Table 1: 2x2 Sensitivity and Specificity for CAV

	SN Pos	SN Neg
RapidSTATUS™ CAV Pos	111	4
RapidSTATUS™ CAV Neg	0	182
Sensitivity	100% (95% CI: 96.7, 100)	
Specificity	97.8% (95% CI: 94.6, 99.4)	

Table 2: 2x2 Sensitivity and Specificity for CDV

	SN Pos	SN Neg
RapidSTATUS™ CDV Pos	73	3
RapidSTATUS™ CDV Neg	3	176
Sensitivity	96.1% (95% CI: 88.9, 99.2)	
Specificity	98.3% (95% CI: 95.2, 99.7)	

Table 3: 2x2 Sensitivity and Specificity for CPV

	HI Pos	HI Neg
RapidSTATUS™ CPV Pos	80	2
RapidSTATUS™ CPV Neg	7	162
Sensitivity	92.0% (95% CI: 84.1, 96.7)	
Specificity	98.8% (95% CI: 95.7, 99.9)	

Based on the 2x2 comparison to the gold standard serological test, the sensitivity and specificity for the RapidSTATUS™ CAV test kit is 100% and 97.8% respectively; for CDV the sensitivity and specificity is 96.1% and 98.3%; and for CPV the sensitivity and specificity is 92% and 98.8%.

Citations:

- [1] M. Taguchi, K. Namikawa, T. Maruo, K. Orito, J. Lynch and H. Sahara, "Antibody titers for canine parvovirus type-2, canine distemper virus, and canine adenovirus type-1 in adult household dogs," *Can Vet J*, vol. 52, no. 9, p. 983–986, 2011.
- [2] O. Bulut, O. Yapici, O. Avci, A. Simsek, K. Atli, I. Dik, S. Yavru, S. Hasircioglu, M. Kale and N. Mamak, "The Serological and Virological Investigation of Canine Adenovirus Infection on the Dogs," *The Scientific World Journal*, p. 6, 2013.
- [3] M. J. G. Appel, L. E. Carmichael, E. P. Bass. CAV-2: a replacement vaccine for canine hepatitis vaccine. *Norden News*. 1973;53:4-6.
- [4] "Canine Distemper," American Veterinary Medical Association, 2019. [Online].
- [5] S. Nandi and M. Kumar, "Canine Parvovirus: Current Perspective," *Indian J. Virol*, vol. 21, no. 1, pp. 31-44, 2010.
- [6] "Canine Parvovirus," American Veterinary Medical Association, 2019. [Online].
- [7] AAHA Canine Vaccination Guidelines: 2017 (updated February 3, 2018): available online at: www.aaha.org.
- [8] M. J. Day, M. C. Horzinek, R. D. Schultz and R. A. Squires, "Guidelines for Vaccination of Dogs and Cats," *Journal of Small Animal Practice*, vol. 57, pp. E1-E45, 2016.
- [9] D. Mouzin, M. Lorenzen, J. Haworth, V. King. Duration of serologic response to five viral antigens in dogs. *J Am Vet Med Assoc*. 2004 1;224(1):55-60.
- [10] J. S. Oh, G. W. Ha, Y. S. Cho, et. al. One-Step Immunochromatography Assay Kit for Detecting Antibodies to Canine Parvovirus. *Clin Vaccine Immunol*. 2006 Apr;13(4):520-4.
- [11] M. Martinez-Gutierrez, J. Ruiz-Saenz. Diversity of susceptible hosts in canine distemper virus infection:a systematic review and data synthesis. *BMC Vet Res*. 2016, 12;12-78.

- [12] K. Mende, B. Stuetzer, U. Truyen, K. Hartmann. Evaluation of an in-house dot enzyme-linked immunosorbent assay to detect antibodies against feline panleukopenia virus. *J Feline Med Surg.* 2014;16(10):805-11.
- [13] A. L. Litster, B. Pressler, A. Volpe, E. Dubovi. Accuracy of a point-of-care ELISA test kit for predicting the presence of protective canine parvovirus and canine distemper virus antibody concentration in dogs. *Vet J.* 2012; 193(2):363-6.
- [14] R. L. Hu, G. Huang, W. Qiu, Z. H. Zhong, X. Z. Xia, Z. Yin. Detection and differentiation of CAV-1 and CAV-2 by polymerase chain reaction. *Vet Res Commun.* 2001; 25(1):77-84.