

PROCEDURES PRO

NASAL SWABS TO DETECT CANINE INFLUENZA VIRUS

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Nasal swabbing is the preferred antemortem sample collection technique for diagnosing acute influenza infection caused by canine influenza virus.



Although oropharyngeal swabs have been recommended in several species,¹⁻³ nasopharyngeal swabs are as or more likely to identify influenza-infected animals and are the current standard for sample collection in multiple species (eg, humans, pigs).⁴⁻⁹ However, nasal swabs are preferred over nasopharyngeal or oropharyngeal swabs in dogs because of the inherent difficulty of obtaining high-quality swabs from the pharyngeal region caused by poor visibility of the target area, especially in large or brachycephalic breeds. In addition, the enhanced sensitivity of newer reverse transcription polymerase chain reaction (RT-PCR)-based influenza assays enable detection of small quantities of virus. Nasal swabs are also safer, as the dog can be muzzled during the procedure.

Swabs can be used to detect influenza via polymerase chain reaction (PCR), virus isolation, and/or antigen-based enzyme-linked immunosorbent assay (ELISA) testing several days before development of a systemic immune response identifiable via traditional serologic methods; however, false-negative samples are common, even

in dogs that later demonstrate influenza antibodies. False-negative results are a major problem with nasal swabs because of these factors:

- ▶ Suboptimal technique
- ▶ Virus inactivation and/or degradation due to improper swab and/or transport media selection
- ▶ Lack of patient compliance during the procedure
- ▶ Poor sample handling
- ▶ Timing of sample collection relative to peak virus shedding

Additionally, as with the recent H3N2 canine influenza virus (CIV) outbreak, false-negative results may occur when a new strain of influenza emerges. Properly collected nasal swabs can be used for virus isolation, regardless of influenza type, allowing for detection and subsequent characterization of new virus isolates through sequencing and development of PCR assays. This decreases the time needed for diagnostic tests for a given influenza strain and to select an updated vaccine based on the newly identified strain(s).

Influenza replication occurs almost exclusively in epithelial cells lining the respiratory tract. Therefore, although swabs of the external nares may be useful for detecting diseases such as leishmaniasis, in which the agent lives in the mucocutaneous region,^{1,10} influenza sampling necessitates a much deeper swab technique to collect virus. To optimize CIV detection, swabs of the distal nasal cavity should be obtained.

Swabs

Various types of nasal swabs are available; selecting an inappropriate type or material can have deleterious effects on

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Restraint is critical.

the procedure and/or test outcome.^{4,5,7,8,11} Because the majority of patient samples will first be tested by PCR and then undergo testing with a virus isolation assay, only swabs acceptable for both diagnostic tests should be chosen. Sterile swabs should be used and may include:

- ▶ Virus transport medium swabs
- ▶ Polyester: standard or flocked-style swabs⁴
- ▶ Culture swab systems for bacteria (only those with liquid media, not agar/gel)^{12,13}
- ▶ Other synthetic swabs; polyurethane foam swabs⁴

The US Centers for Disease Control and Prevention (CDC) does not recommend cotton or calcium alginate swabs for influenza diagnosis because of potential virus degradation and/or PCR interference.¹⁴

Avoid using wooden, paper, or aluminum wire handles because of the potential for residual chemicals to inactivate and/or degrade viral particles collected on the swab.¹⁵ Wooden handles are also more prone to break. Wire handles tend to bend during insertion, especially if the animal is not adequately restrained and the swab head is too small to adequately contact nasal epithelium; this can lead to poor virus recovery. Wire swabs may be useful, however, in pediatric or small-breed patients, in which small nasal passages may hinder intro-

duction of a larger-diameter swab without significant trauma. Do not use wooden-handled swabs on live animals.

Restraint

Restraint is critical to prevent a swab from breaking in the nasal passage. It may take several veterinary team members to adequately restrain an unsedated dog to ensure its head remains still during swabbing. A swab that breaks off in the nasal passage can often be removed with a hemostat. Sedation should be used with caution in canine patients exhibiting respiratory compromise and/or distress.

WHAT YOU WILL NEED

- ▶ Sterile 6-inch polyester (or similar) swabs with plastic handles
- ▶ Sterile 5-mL red top, snap cap, or similar tube
- ▶ Sterile isotonic saline or phosphate-buffered saline solution
- ▶ Open-ended muzzle (not shown)
- ▶ Sedative drugs (may be contraindicated)



CIV canine influenza virus

ELISA enzyme linked immunosorbent assay

PCR polymerase chain reaction

RT-PCR reverse transcription polymerase chain reaction

STEP-BY-STEP NASAL SWAB TECHNIQUE

STEP 1

With the dog muzzled,* place the swab parallel to the dog's nose so that it extends to the medial canthus of the eye (*Figure 1*). This is the maximum depth the swab can be inserted into the nasal cavity. This distance varies with the length of the dog's nose.

*Muzzle was removed for photographs to provide better visualization.

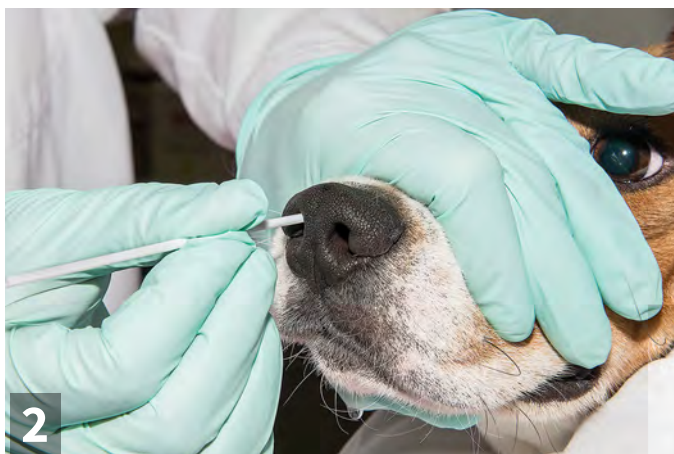


Author Insight

Avoid premoistening the swab, as this limits absorptive capacity and can result in lower sample recovery.

STEP 2

Hold the stick portion of the swab at the measured length to ensure the swab has been inserted as far as possible (*Figure 2*), which maximizes contact with the respiratory epithelium. To help minimize mucosal damage, quickly but gently twist the swab around its axis as it is advanced into the nasal cavity. Direct the swab upward and toward the midline to minimize trauma as it passes through the external nares into the nasal passages. Inserting the swab takes a fair amount of pressure, so speed is essential to minimize patient irritation and minimize the potential for swab breakage if the patient is not adequately restrained.

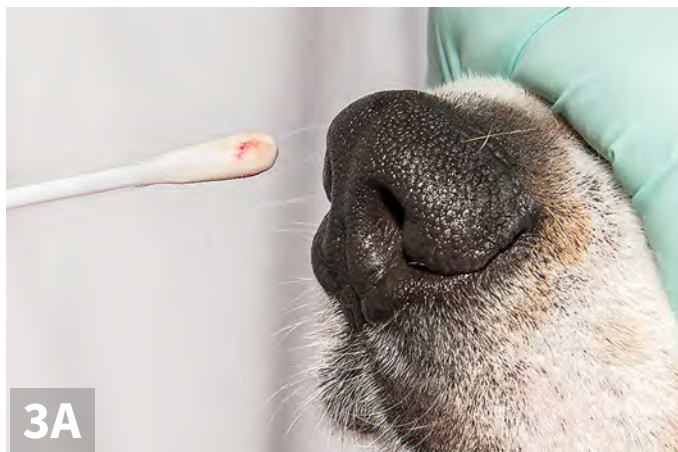


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Never reuse a bent swab.

STEP 3

Swab both nasal passages; the same swab can be used for both sides, although this may increase the risk for handle breakage. If 2 swabs are used, they can be combined in the same tube. A small amount of blood on the swab is normal (*Figure 3A*), and larger amounts may be seen due to inflammation of the nasal epithelium (*Figure 3B*). If a large amount of blood is present on a single swab, place that swab in a tube by itself. Most dogs will have a small amount of blood-tinged discharge (*Figure 3C*) and may sneeze blood intermittently following swabbing. Epistaxis may also occur for several minutes after the procedure but is usually self-limiting.

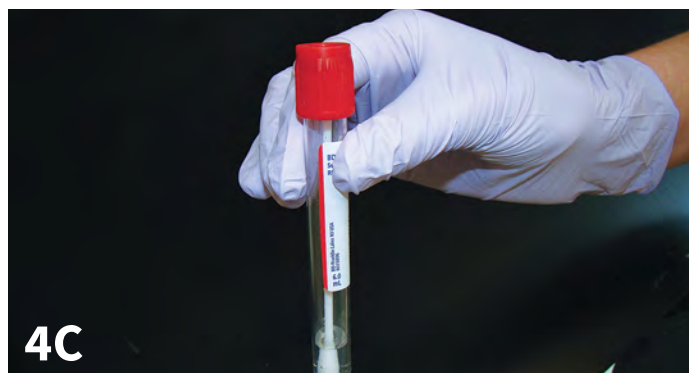


When inserting the swab, speed is essential to minimize patient irritation.

STEP 4

Place the swab into a sterile red-top or similar sterile leak-proof container (*Figure 4A*). To close the cap, snap off the swab handle by inserting the swab into the tube until it is 1 to 2 cm from the bottom of the tube and folding the handle over the lip of the tube as shown (*Figure 4B*).² Cap the tube, making sure the swab handle is not contacting the cap, as this can cause leakage. If the handle is too long, shorten it as needed. Add ≈1 mL of sterile physiologic saline solution (eg, surgical saline, 0.9% IV saline solution) to the tube to help prevent sample desiccation if a commercial virus or bacteria collection system that contains a liquid medium is not used; ensure the entire swab tip is submerged in liquid (*Figure 4C*). When using virus transport or sterile culture swabs, the swab handle does not need to be shortened and should be inserted into the included media tube as-is.

Make sure the swab handle does not contact the cap.



STEP 5

Store the sample(s) on ice (not dry ice) or at 39.2°F (4°C) in a refrigerator immediately after collection (**Figure 5**). Ship the samples on ice packs overnight to the receiving laboratory—the same day if possible and preferably no later than the next day. Do not freeze samples, as freezing can hinder recovery of live virus. Samples may be refrigerated for several days then shipped if necessary, but this can increase the potential of a false-negative virus isolation test result. Because RT-PCR is generally unaffected, samples can be tested this way in a majority of cases, regardless of shipping delays. Ensure the recipient laboratory is testing for both the H3N8 and H3N2 influenza A strains.



All tools, equipment, and contact surfaces (eg, muzzles, examination tables) should be disinfected appropriately after use on suspected influenza cases to prevent potential virus transmission to subsequent patients.

MORE ABOUT INFLUENZA

Clinical influenza can be caused by many virus subtypes, all with unique species predilections. Crossover and subsequent adaptation of viruses among species remains an ongoing concern, although current canine influenza variants do not appear to readily cross back to other species.

Influenza variants and hosts include:

- ▶ Influenza A virus: Humans, pigs, horses, dogs, cats, birds, ferrets, guinea pigs, mice, others
- ▶ Influenza B virus: Humans, ferrets, seals
- ▶ Influenza C virus: Humans, dogs, pigs, cows (also suggested as influenza D in cattle)¹⁶

Influenza A is further subdivided based on hemagglutinin (HA) and neuraminidase (NA) receptor types. Currently, 18 HA and 11 NA variants have been identified that can be combined in innumerable ways to yield unique viruses. Historically, H3N8 viruses originated in horses and spread to dogs, but the most recent canine virus, an H3N2 variant, appears to be of avian origin.¹⁷ It originated in 2007 in South Korea, where it was observed to infect dogs and cats; it has infected both species in the United States.¹⁸ Experimentally, dogs have been readily infected with both H5N1 and H6N1 avian-origin viruses. This indicates the potential for further influenza subtype introductions into the canine population.¹⁹

HA hemagglutinin
NA neuraminidase
RT PCR reverse transcription
polymerase chain reaction

Conclusion

Nasal swabbing is an excellent diagnostic tool for identifying acute influenza infections, but it requires the right combination of swabs and technique to yield high-quality samples suitable for PCR, virus isolation, and other diagnostic tests.

Animal use was conducted under approval by the Animal Care and Use Committee of the University of Georgia, an institution accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

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PCR polymerase chain reaction