# Collection of Specimens from Swine for the Detection of Influenza A Virus by Molecular Assays or Virus Isolation

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## Recommended optimum specimens for detection of influenza A virus from swine:

1. Nasal swab
2. Lung tissue (post mortem)
3. Tracheal swab (post mortem)

### 1. Nasal swab

DO NOT pool swabs from individual pigs.

Use a sterile polyester (Dacron) or polyurethane foam tip swab or flocked swab (e.g. a swab that has a tip coated with short nylon fibers that are arranged in perpendicular fashion) in virus transport medium with a standard or fine (very flexible and does not break readily) plastic shaft at least 6 inch/15 cm in length.

A. The pig should be properly restrained with the head positioned upward to allow easy access to the nasal cavity. Anesthesia is not needed.

B. Insert the sterile swab carefully around the nasal protuberance and into the nasal cavity in a dorsal-medial direction. Gently swab the surface of the nasal mucosa using a circular motion to cover as much of the nasal mucosal surface as possible. The swabbing procedure should occur for approximately 5 seconds to allow for absorption of mucus. The swab will collect nasal mucosal secretions and surface epithelium.

- Ensure the nostril is free of dirt, feces and extraneous materials like feed particles
- Avoid touching the skin with the swab as you enter the nasal cavity
- It is important not to scrape too hard, as drawing blood is undesirable. If the pig struggles and resists the restraints, release the swab and leave it in the nostril. To avoid injury, do not forcibly remove or insert the swab.
- Using the same swab, remove the swab from one nostril and repeat the same procedure in the other nostril
- Approximate depth to insert swabs for optimal sample:
    - 1 cm for piglets 0 to 4 weeks-old
    - 2 cm for nursery pigs from 4 to 7 weeks-old
    - 3 to 4 cm for fattening pigs > 7 weeks of age
    - Above depth is optimal when collecting sample from sick pigs with respiratory symptoms, and twice deeper insert would result in better virus isolation when trying to isolate the virus from asymptomatic pigs.

C. Once the nasal swab has been collected, vigorously mix the swab in a transport liquid medium designed for maintaining viruses (viral transport medium or PBS), if possible, and place into a polypropylene tube. Dry swabs can also be used, but virus inactivation may occur more readily.

D. The volume of viral transport medium should be sufficient to cover the head of the swab. For details on viral transport medium, refer to the publication by Jianqiang Zhang and Phillip C. Gauger, “Isolation of Swine Influenza Virus in Cell Cultures and Embryonated Chicken Eggs.“ (2014) In: Spackman E. (Ed.) Animal Influenza Virus. Methods in Molecular Biology (Methods and Protocols), Vol 1161. Pages 265-276 Humana Press, New York, NY

E. To remove the swab handle, back the swab out of the tube slightly and bend the handle back and forth over the edge of the tube until it breaks (some shafts have a

breaking point). Alternatively, tools such as scissors or wire cutters can be used to cut the swab handle but care must be taken to avoid contamination of samples with improperly disinfected tools.

F. The swab handle should be short enough to allow the tube to close tightly and long enough to allow for easy retrieval once the tube reaches the laboratory.

G. Clearly label with appropriate identification (ID) and immediately refrigerate or chill.

H. Store on wet ice (at approximately 4°C) and ship refrigerated (if possible, especially if viral transport medium is not used) in an upright position to reduce chances of leakage.

- It is important for the specimen to remain cold, at least from the collection to the shipping process. A constant cold chain should be strictly maintained if the swab is not placed into a viral transport medium.
- Avoid any freezing-thawing cycle before the swab reaches the lab.

I. Do not pool swabs from more than one pig into a single tube.

## 2. Lung Tissue:

A. Collect multiple sections of lung tissue from areas with lesions (recognized by Veterinarian/Pathologist) or at the junction of healthy lung and areas of lung with lesions. Visible lesions may include clearly demarcated areas of pneumonia that appear collapsed or consolidated, slightly firm and purplish-red. Healthy/normal areas of lung are pale pink and soft and should only be sampled if there is no evidence of pneumonia.

Fresh lung tissue should be collected as autolysis will cause the production of enzymes which can inactivate virus or be inhibitory to PCR.

If collection of a lung swab, rather than tissue, is desired, thrust or plunge the swab into the excised portion of lung and vigorously swab the lung tissue. Place the swab in a 2.0 mL sterile vial with 1.0-1.5 mL of viral transport media (or PBS) and mix inside the vial. Break off the swab as described above or remove the swab and close the vial.

If collection of a bronchial swab, rather than a lung swab, is desired, carefully open the bronchus with sterile scissors and vigorously swab the bronchial lumen. Place the swab in a 2.0 mL sterile vial with 1.0-1.5 mL of viral transport media (or PBS) and

mix inside the vial. Break off the swab as described above or remove the swab and close the vial.

B. Lung tissue samples should be at least 3.0 cm in diameter or approximately 3-4 grams.

C. Double bag and clearly label the collected lung tissue samples with appropriate ID and refrigerate or chill immediately. For preservation of the specimen it is necessary to maintain a cold chain from the point of collection through shipping and receipt of the testing laboratory.

E. Do not pool tissues from more than one pig in a bag. Lung tissue from each individual animal should be packaged separately.

## 3. Tracheal Swab

A. Swine influenza virus can be detected in post mortem by collection of tracheal swab. This is especially important for virus that replicates in the lower respiratory tract.

B. The trachea is at the junction of the upper and lower respiratory passage.

C. Using sterile Dacron/Nylon or polyester swab, vigorously swab the surface of the tracheal mucosa to collect mucosal secretions and surface epithelium.

D. Once the tracheal swab has been collected, vigorously mix the swab in a transport medium designed for maintaining viruses (viral transport medium or PBS).

E. The volume of viral transport medium should be sufficient to cover the head of the swab.

F. To remove the swab handle, back the swab out of the tube slightly and bend the handle back and forth over the edge of the tube until it breaks. Alternatively, tools such as scissors or wire cutters can be used to cut the swab handle but care must be taken to avoid contamination of samples with improperly disinfected tools.

G. The swab handle should be short enough to allow the tube to close tightly and long enough to allow for easy retrieval once the tube reaches the laboratory.

H. Clearly label with appropriate ID and immediately refrigerate or chill.

- It is important for the specimen to remain cold (approximately 4°C) from the collection to the shipping process. A constant cold chain should be maintained.

I. Store on wet ice (at approximately $4^0$C) and ship refrigerated in an upright position to reduce chances of leakage.

J. Do not pool swabs from more than one pig into a single tube.

### Other possible specimens from swine:

Other specimens suitable from swine for detection of influenza A virus include oral fluids, bronchio-alveolar lavage fluid (BALF), bronchial swabs, lung swabs, environmental surfaces, and air.

### References:


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