



### **Weekly pool sampling (“bucket sampling”) in poultry species that often do not manifest clinical signs when infected with HPAI**

To improve early detection of infection in poultry within the surveillance zone, the clinical inspection of poultry species/categories that may not display significant clinical signs when infected with HPAI (e.g.: ducks, geese or broiler chickens) should be complemented by collecting dead birds, or samples taken from them, to be pooled and tested weekly.

The recommended surveillance strategy is the pooled sampling of dead birds (with a maximum of 20) over a period of about one week, equally subdivided from each shed of the holding of the species cited above in the surveillance zone (during the length the surveillance zone is in place). This strategy will enable to detect the infection between 4 and 12 days post-virus introduction.

In order to form the pool sampling, up to five carcasses/samples of birds deceased on the day of sampling should be collected crossing each shed starting from one corner and going diagonally back and forth in the opposite direction forming an X. The remaining carcasses/samples should be uniformly collected from dead birds stored frozen from the preceding 3-5 days (it is recommended to agree with the farmer to take the carcasses out of the freezer the day before the visit to the farm to work on thawed carcasses).

#### ***Sampling options:***

- Whole carcasses of dead birds adequately transported to the diagnostic laboratories for the detection of HPAI viruses.
- Alternatively, heads and necks may be separated from the carcasses and transported to diagnostic laboratories so that brains and/or trachea (or tracheal swabs) are examined for HPAI.
- Another option could be the collection of tracheal and/or cloacal swabs from the carcasses. As soon as the growth characteristics of the virus are known, the competent authority may decide to choose either tracheal or cloacal swabs rather than to collect both depending on whether the virus replicates better in the respiratory or gastrointestinal tract and also taking into account the species concerned. A pool of up to five swabs of each type should be run.

Collection, transport and examination of carcasses /samples must be performed according to the guidelines available on the EURL AI-ND website (<https://www.izsvenezie.com/reference-laboratories/avian-influenza-newcastle-disease/diagnostic-protocols/>).