

# Sampling protocol and deliver to the laboratory for successful isolation of poultry mycoplasma species

## Introduction

Agents belonging to **Mycoplasma spp. are considered fastidious microorganisms** because their growth in specific and selective media takes long time. Moreover, contaminant bacteria could compromise Mycoplasma growth in vitro.

**Culture isolation is used mainly to confirm a specific diagnostic suspect of mycoplasmosis** and also provides a unique opportunity to keep live microorganisms and learn more about them such as their antimicrobial susceptibility (i.e. Minimum Inhibitory Concentration).

The **M.I.C. provides practitioners with a useful tool in the choice and management of appropriate therapy for affected animals**, moreover the live strains can also be used for genotyping, animal trials and other laboratory activities.

Based on these brief considerations, the following recommendations should be applied in order to enhance the chance of mycoplasmas isolation.

## When to sample

**The acute phase of disease (until the 30th day post infection) should be considered the best period for sampling.**

The practitioners should focus their attention on symptomatic animals **and be aware if any drug treatment has been applied previously** in the flocks. Antibiotics such as macrolides, tetracycline, fluoroquinolones and aminoglycosides can have adverse impact on mycoplasma isolation. It is better to avoid the sampling of treated birds.

## What to sample

**The tracheal swab is considered the best sample in live animals for most mycoplasma species**, whereas the cloacal or phallus swab is recommended for a suspected *Mycoplasma meleagridis* or *Mycoplasma iowae* infection.

Instead, **when dead birds are available**, mycoplasma isolation can be performed with swabs from different tissues/organs: upper-middle trachea, lungs, air sacs, oviduct, joints and cloaca.

**Swabs of the yolk sac** should be collected during the last third of the egg incubation period in the specific case of decreased hatchability of embryonated eggs referable to mycoplasma infection (i.e. after the 15th and the 20th day of incubation in chickens and in turkeys, respectively).

If you have any particular requests or concerns, please do not hesitate to **contact the laboratory**. We will be glad to support you in management of your specific situation.

## Sample size

**During the acute phase in a symptomatic group**, 10 tracheal swabs are considered suitable for a successful isolation of the strain involved in the outbreak.

**During the chronic phase**, the sample size should be increased up to 30 tracheal swabs; furthermore it could be helpful to test these specimens by PCR methods.

**In case of decreased hatchability**, referable to mycoplasma infection, 60 eggs with dead embryos (in the late phase of egg incubation) could be enough to detect the pathogen. These samples can be tested initially by biomolecular techniques and the culture method will eventually be applied only for positive PCR samples.

If you have any particular requests or concerns, please do not hesitate to **contact the laboratory**. We will be glad to support you in management of your specific situation.

## How to sample

**The protocol of sampling is the most important and critical factor in the entire procedure of isolation.** The first cause of failure on mycoplasma isolation is the contamination due to the slow growth of avian mycoplasma compare to other bacteria or fungi.

To avoid any contamination of the specimens, sampling should be performed in accordance with the following recommendations.

First of all, it is really important to **use the smallest possible swab** (urethral swab straight aluminium wire with rayon tip) and run it in and out of the trachea avoiding any contact with the surrounding oral mucosa. If contact occurs, please change the swab.

In **dead animals swabs from several tissues** (see "What to sample" section) can be collected after cauterizing the tissue surfaces. Remember that mycoplasmas live in the epithelial surface of infected tissue and for this reason it is important to sample the epithelial surface.

Then the **samples are mixed vigorously into the liquid transport medium for at least 5 seconds**, before being removed and placed in their case for the storage. In each test tube inoculate a maximum of 5 swabs, one per animal. Give the same identification number to the swab case and the broth medium.

## How to store and deliver the sample

Once the samples are collected, **deliver it as soon as possible** to ensure the best results in mycoplasmas isolation.

The transport medium **can be refrigerated if the sample arrives to the lab in 24 h maximum**, but if it is not possible it should be kept in dry ice or at -20°C. Please avoid any thermic shock to the samples, for not compromise the isolation procedure.

If you have any particular requests or concerns regarding transport and delivery, please do not hesitate to **contact the laboratory**.

## How to store and deliver the isolates

The Mycoplasma isolates **could be stored in lyophilized form and kept at +4°C** until their arrival at the laboratory. Alternatively, specific agreement shall be taken between the laboratorists involved.

If you have any particular requests or concerns regarding transport and delivery of live strains, please do not hesitate to **contact the laboratory**.

## Contacts

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