



THE IMPROVEMENT OF MYCOPLASMOSIS CONTROL USING THE AVIAN MYCOPLASMA PCR TECHNOLOGY

Avian mycoplasmosis: limits of the current diagnosis methods

Mycoplasmosis (caused by *Mycoplasma gallisepticum* (MG), *M. synoviae* (MS), *M. meleagridis* (MM) and *M. iowae* (MI)) is a wide spread disease affecting poultry production all over the world. International poultry breeding companies are making a great effort to eradicate mycoplasma from their primary breeding stocks. Nevertheless, the control of mycoplasmosis in parent breeders and poultry production flocks still remains a problem in some countries.

The diagnosis of avian mycoplasma is mainly based on culture and serology. But, culture is long and tedious and mycoplasma isolation can suffer from contamination by fast growing micro-organisms. The most used serological test, the Rapid Slide Agglutination (RSA) often lacks of specificity, especially at the time of vaccination with inactivated vaccines. For all of these reasons, Labofarm has developed molecular methods for avian mycoplasma detection and characterization.

PCR technology

PCR (Polymerase Chain Reaction) is a patented technology and a laboratory process in which a specific DNA segment from a mixture of DNA chains is rapidly replicated, producing a large amount of detectable DNA.

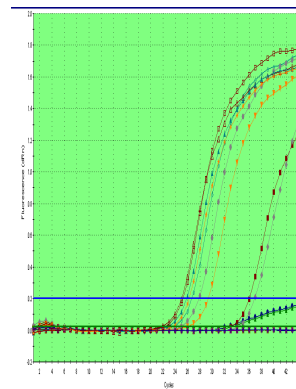


PCR (Polymerase Chain Reaction) is a patented technology and a laboratory process in which a specific DNA segment from a mixture of DNA chains is rapidly replicated, producing a large amount of detectable DNA. Using this method, the detection of a particular micro-organism (virus or bacteria) or specific gene (virulence factor) in a sample is very fast (in a few hours), very specific and very sensitive (only one copy of the suspected

target gene theoretically gives a positive result). More interesting is the fact that positive individual can be detected from the first day of infection. The PCR is now applied for the detection of avian mycoplasma in poultry and poultry farms environment.

Avian mycoplasma PCR test

The most useful PCR test is the real time multiplex PCR test, enabling concomitant detection of MG and MS in the same sample, at the same time. Moreover, the use of an internal control (DNA fragment added in each sample) is a guarantee of good processing. Additional tests for MM and/or MI detection can also be run.



The specificity of such a test is demonstrated in that no amplification is detected with other mycoplasmas or with any bacteria species currently found in poultry. Consequently, mycoplasma can be detected even if the samples are heavily contaminated by other bacteria.

The veterinarian or the poultry farming staff can take advantage of this technology in the mycoplasma diagnosis field, especially in terms of rapidity of getting the answer. The total duration of the process takes less than 6 hours. In practice, for any sample received by the diagnostic laboratory before 10.00 am, one can get a result at 18.00 pm, enabling rapid decision concerning the tested birds.

The samples and the sampling size

As for any diagnostic test, the samples must be representative of the flock and of the pathology, because they must be likely to contain the microorganisms. The easiest sample remains the trachealswab using plastic applicators cotton swabs.



At the time of sampling, the swab is introduced in the trachea of the bird, moved up and down two to three times and

put in Amies charcoal medium. Amies charcoal medium is a suitable medium preventing the sample from desiccation and allowing good transport conditions. The swabs have to be brought directly to the laboratory or sent by rapid post or any express transport. The samples must be refrigerated during their transport to the laboratory to avoid DNA lyses. To our experience, the time taken between sampling and swab processing in the laboratory can reach up to 5 days at +4-8°C without reducing the chance of getting PCR positive results. Airsac swabs, taken at necropsy, are also good samples for mycoplasma recovery by PCR and rag-swabs, realized on the building walls or material give representative results of the mycoplasma contamination level in the environment of the poultry farm.

The number of swabs needed to detect mycoplasma in a flock depends on the expected prevalence, the level of confidence and the total number of birds in the flock.

In practice, the sampling size depends on whether there is a mycoplasmosis suspicion or not. In the case of a mycoplasmosis suspicion, 20 to 30 individual tracheal swabs, taken on suspicious birds, allows the detection of the pathogen. In a routine control, 40 to 60 individual swabs, depending on whether the level of confidence is high or low, are needed.

How to use mycoplasma PCR ?

1- Routine sanitary control

Mycoplasma PCR is used in the case of routine mycoplasma sanitary control of the turkey or *Gallus* breeders. Sampling size and frequency have then to be determined between the veterinarian in charge of the company and the laboratory, according to the official poultry managing program of the State.

2 -Sanitary status/transfer follow up

The PCR method is also very useful to check a mycoplasma free flock in a mycoplasma contaminated area, or a poultry batch before transfer to another farm, avoiding therefore the possibility of a cross contamination.

3 – False positive RSA

It is well known that RSA can give false positive results, especially at the time of vaccination with inactivated vaccines. 20 to 30 tracheal swabs tested by PCR is then a rapid and specific way to confirm or not the doubtful serological results.

4 – Early detection

As culture is long and antibody response detectable by RSA can take several days, birds with slight clinical signs should be tested by PCR. Mycoplasmosis confirmation is achieved within a day allowing rapid antibiotic therapy.

5 – Environmental contamination

PCR realized on drag-swab seems us to be a good tool to check disinfection between two successive flocks in the case of a mycoplasma contamination.

How many samples should I take ?

Hypothetic prevalence	5%	5%	20%	20%
	Routine control program		Low clinical signs	
Flock size	5000-10000	5000-10000	5000-10000	5000-10000
Level of confidence	95%	99%	95%	99%
Sampling size	60	90	15	20

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