

Mycoplasma Diagnostics

POULTRY DIAGNOSTIC & RESEARCH CENTER | UNIVERSITY OF GEORGIA | ATHENS, GA

Avian mycoplasmosis presents a persistent and significant challenge to global poultry production, primarily due to the economic impact associated with morbidity and mortality. Effective disease mitigation is contingent upon stringent biosecurity protocols and robust, systematic surveillance supported by reliable diagnostic assays.

This document outlines the diagnostic capabilities provided by the UGA–PDRC Diagnostic Laboratory for all economically consequential *Mycoplasma* species and specifies the critical requirements for sample collection and handling necessary to ensure test accuracy.

Diagnostic Methodology

The UGA–PDRC Diagnostic Laboratory provides comprehensive testing for the four principal avian *Mycoplasma* pathogens: *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), *Mycoplasma meleagridis*, and *Mycoplasma iowae*.

Pathogen	Primary Diagnostic Methods	Notes
MG and MS	Serology, Culture, Polymerase Chain Reaction (PCR)	Standardized assays for surveillance and clinical diagnosis.
<i>M. meleagridis</i>	Culture, PCR	Serological testing is available but infrequently requested.
<i>M. iowae</i>	Culture, PCR	No validated serological assays are available.



Sample Integrity Requirements and Handling Protocols

The reliability of diagnostic results is directly proportional to the quality and integrity of the submitted specimens.

Whole Blood and Serum

Sample Type	Collection & Processing	Storage & Submissions	Critical Handling Preclusions
Whole Blood	Collect aseptically. Maintain at room temperature to facilitate clot formation and subsequent serum separation. Centrifugation is recommended for efficient serum separation.	Standardized assays for surveillance and clinical diagnosis.	Exposure to excessive heat or cold must be avoided, as it induces red blood cell lysis (hemolysis), which may compromise sample integrity and invalidate certain assays.
Serum	Separated serum.	Must be refrigerated or stored on cold packs until submission to maintain stability.	Serum intended for plate agglutination assays must not be frozen. Freezing can alter protein structure, leading to non-specific agglutination reactions and false-positive results.

Specimen Collection and Handling

Ensuring specimen integrity is paramount for reliable *Mycoplasma* detection via culture and molecular assays. All shipped samples must be sent via overnight courier services (e.g., FedEx or UPS) to ensure optimal quality upon arrival.

Assay Type	Collection Medium	Handling and Transport	Notes
Culture	Transport swabs (e.g., tracheal/choanal cleft)	Swirl swab in mycoplasma broth medium, discard the swab, and transport medium on ice.	Viability of the organism is required.
PCR Only	Sterile swabs without transport media.	Must be stored and transported at a cold temperature.	Organism viability is not required for molecular detection.



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Tissues

Sample Type	Primary Use	Preferred Species	Handling Requirement
Tracheal Tissue	Most frequently submitted.	<i>M. gallisepticum</i> (MG), <i>M. synoviae</i> (MS), <i>M. meleagridis</i> (MM)	All tissue samples must remain chilled during transport.
Esophageal Tissue	Dead-in-shell embryos, poults.	<i>M. iowae</i> .	All tissue samples must remain chilled during transport.
Additional Samples	Semen, Synovial Fluid	<i>M. iowae</i> (semen), <i>M. synoviae</i> (synovial fluid).	All tissue samples must remain chilled during transport.

Whatman FTA Cards (International Submission)

Application	Handling Requirements	Constraint
International Submissions (Tissue impressions or swabs)	Must be stored in a dry environment and protected from direct sunlight. All necessary documentation should be included for customs.	Fresh tissue or swabs are preferred for domestic cases.



Diagnostic Methods and Interpretation

The UGA–PDRC utilizes a panel of serological and molecular techniques to diagnose *Mycoplasma* infection.

Serological Assays: Serological tests detect the host's antibody response.

Assay	Principle	Sensitivity/Specificity	Key Interpretation
Plate Agglutination	Serum mixed with stained antigen; rapid reaction on glass/ceramic tile.	Highly sensitive early in infection, but prone to false positives.	All positive reactors must be confirmed by Hemagglutination Inhibition (HI).
ELISA	Enzyme-linked immunosorbent assay.	More specific but slightly less sensitive than Plate Agglutination.	Suitable for screening and assessing vaccination responses. Positive results require HI confirmation. (Frozen serum may be used).
HI	Hemagglutination Inhibition Assay	More specific but less sensitive than Plate Agglutination or ELISA (may miss early infections).	HI titers of 1:40 to 1:80 or higher are generally considered positive at PDRC, but interpretation requires flock-specific context. Titers may not be detectable until several weeks post-infection.



Mycoplasma Culture and Isolation

Culture involves isolating viable organisms, which is crucial for subsequent strain identification.

Media: Frey's media is the primary medium for MG and MS isolation. M-ORT media is used specifically for *M. iowae* and *M. meleagridis*

Procedure: Samples are inoculated into broth media and incubated at 37°C for 3–4 weeks. Broth is generally more sensitive than initial plate inoculation.

Identification: Broth cultures are plated onto agar plates after 3–5 days for further isolation and identification. For *M. meleagridis* and *M. iowae*, primary isolation onto agar plates may be more effective than broth.

PCR and Sequencing

PCR is the most rapid and sensitive method for nucleic acid detection.

Detection: Real-time PCR assays are available for MG, MS, *M. iowae*, and *M. meleagridis* at the PDRC lab, providing rapid, highly sensitive, and specific detection of *Mycoplasma* DNA.

Strain Differentiation: Conventional PCR and subsequent sequencing of specific gene targets (e.g., *vlhA* gene for MS) is used to differentiate between field strains and vaccine strains and to support epidemiological comparisons. Strain specific PCR is available to specifically identify some vaccine strains (e.g. ts-11)

DNA Integrity: DNA integrity is essential for molecular diagnostics. Samples must be kept cold, protected from UV exposure, and handled without direct contact.

Conclusion

Accurate diagnosis necessitates strict adherence to proper sample collection, handling, and transport protocols. Clients are encouraged to consult the diagnostic laboratory for guidance regarding specimen preparation or test selection.

Additional resources and submission forms are available on the laboratory website:
<https://vet.uga.edu/diagnostic-service-labs/pdrc-diagnostic-services>

