Avian Mycoplasma – an industry update

Naola Ferguson-Noel, DVM, MAM, PhD
Current Picture

• MG
  – Low prevalence
  – High significance

• MS
  – Higher prevalence
  – Lower significance
Check Tests

- Panel of convalescent chicken sera against *M. synoviae* and *M. gallisepticum*
- Panel of tracheal swabs and purified DNA from *M. synoviae* and *M. gallisepticum* infected chickens
Antisera Check Tests

- 67 “Known” kits
  - 10 MG+
  - 10 MS+
  - 10 negative

- Pass = Score of 80% or higher
Antisera Check Tests Results

Range of Scores = 95-100%

# PASS = 49 (100%)

# FAIL (≤ 70%) = 0
Antisera Check Tests Results

SEROLOGY: PASS  Score = 100%

SPT (Score = 100%)
ELISA – Zoetis Combo (Score = 100%)
HI (Score = 100%)

(Similar results for 3 technicians)

Comments:
• Did not run all samples for MG and MS on SPT or HI

Summary of All Participants (n= 49):
Range of Scores = 95-100%
# PASS = 49
# FAIL (≤ 70%) = 0
PCR Check Tests

• 62 Kits
  – 41 “Blind”
  – 21 “Known”

• Pass = Score of 80% or higher
PCR Check Tests

• 5 swabs pools (5 swabs each)
  – 1 negative
  – 1 MG+ (weak)
  – 1 MS+
  – 2 MG and MS+ (1 weak)

• 5 DNA samples
  – 1 negative
  – 1 MG+ (weak)
  – 1 MS+
  – 2 MG and MS+ (1 weak)
PCR Check Tests

• Strong positive Ct = 20 - 27

• Weak positive Ct = 30 - 35
PCR Check Tests Results

Swabs:
Range of Scores = 70-100%
# PASS = 53 (93%)
# FAIL = 4
PCR Check Tests Results

DNA:
Range of Scores = 70-100%
# PASS = 53 (98%)
# FAIL = 1
PCR Check Test Results

2) PCR (Blind Kit):

Swabs: **PASS**  Score = 90%

DNA: **PASS**  Score = 100%

<table>
<thead>
<tr>
<th>Blind Panel</th>
<th>Expected Results (Your deviation from expected highlighted)</th>
<th>% Participants with correct result (Av. CT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MG</td>
<td>MS</td>
</tr>
<tr>
<td>Swab label</td>
<td></td>
<td></td>
</tr>
<tr>
<td>101</td>
<td>Weak Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>102</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>103</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>104</td>
<td>Positive</td>
<td>Weak Positive</td>
</tr>
<tr>
<td><strong>105</strong></td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>DNA tube</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Weak Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
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<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>Positive</td>
<td>Weak Positive</td>
</tr>
<tr>
<td>5</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

* False positives

Positive expected Ct = 20 - 35
Weak Positive expected Ct = 30 - 35

Comments:
- Incorrect results in submission **highlighted**
PCR Check Test Results

DNA
MG - True Positives

<table>
<thead>
<tr>
<th>DNA</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA 2</td>
<td>96</td>
</tr>
<tr>
<td>DNA 4</td>
<td>100</td>
</tr>
<tr>
<td>DNA 5</td>
<td>98</td>
</tr>
</tbody>
</table>

Av Ct

31.1
21.8
23
PCR Check Test Results

Swabs - MG

Ct value

Submission No.
PCR Check Test Results

DNA - MG

Ct value

Submission No.
PCR Check Test Results

Extraction Procedures

- Boiling
- Column Kits (Qiagen)
- Magnetic Bead (Magmax/Kingfisher)
PCR Check Test Results

Magnetic Bead Extraction Volumes

- 300ul
- 200ul
- 175ul
- 150ul
- 100ul
- 50ul
- ?

Values: 0, 1, 2, 3, 4, 5, 6, 7
Antisera Kits

* Required

Company Name *

Your answer

Contact Information of Requestor (name, email, & phone) *

Your answer

Contact Information of Lab Manager (name, email, & phone) *

Your answer

Additional Contact Information of all Lab Members Who Will be Involved (name & email) *

Your answer

Fax # *

Your answer

Antisera Kit (Blind) *

Choose

Antisera Kit (Known) *

Choose

PCR Kit (Blind) *

Choose

Next panel – August/September 2017
PCR

- Swab material
- Moistened vs Dry swabs
- Temperature and time of storage
- Transport/Prep media
Swabbing for PCR
Swabbing for MS Detection by Real-Time PCR

- Oropharyngeal
- Choanal Cleft
- Tracheal

Mean (Genome) Copy No. Log10

2.0 2.1 2.2 2.3 2.4 2.5 2.6 2.7 2.8
Swabbing for MG and MS Detection by Real-Time PCR
Swabbing for MG and MS Detection by Real-Time PCR
Thank you

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