

Supplemental data

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An Affordable Detection System based on RT-LAMP and DNA-Nanoprobes for Avian Metapneumovirus

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Supplemental Table S1-A: Summary of the comparative analysis for detection of aMPV in different type of samples. Samples tested as negative but positive for Infectious Bronchitis virus (IBV) or Infectious Laryngotracheitis Virus (ILT) are in parentheses.

| Sample code | Specie | Type of sample | RT-qPCR | RT-LAMP/ DNA-nanoprobes |
|-------------|---------|-----------------|---------|----------------------------|
| M1 | Turkey | Tracheal tissue | + | + |
| M2 | Turkey | Pharyngeal swab | + | + |
| M3 | Turkey | Tracheal tissue | + | + |
| M4 | Turkey | Pharyngeal swab | + | + |
| M5 | Chicken | Pharyngeal swab | + | + |
| M6 | Chicken | Pharyngeal swab | + | + |
| M7 | Chicken | Tracheal tissue | + | + |
| M8 | Chicken | Tracheal tissue | - (IBV) | - |
| M9 | Chicken | Tracheal tissue | + | - |
| M10 | Chicken | Tracheal tissue | + | + |
| M11 | Chicken | Tracheal tissue | + | + |
| M12 | Turkey | Tracheal tissue | + | + |
| M13 | Turkey | Tracheal tissue | + | + |
| M14 | Chicken | Pharyngeal swab | - (IBV) | - |
| M15 | Chicken | Pharyngeal swab | - (ILT) | - |
| M16 | Chicken | Pharyngeal swab | + | + |
| M17 | Chicken | Tracheal tissue | - (ILT) | - |
| M18 | Chicken | Pharyngeal swab | + | + |
| M19 | Chicken | Pharyngeal swab | + | + |
| M20 | Turkey | Tracheal tissue | + | + |
| M21 | Turkey | Tracheal tissue | + | + |
| M22 | Turkey | Pharyngeal swab | + | + |
| M23 | Turkey | Tracheal tissue | + | + |

| | | | | |
|-----|---------|-----------------|---------|---|
| M24 | Turkey | Tracheal tissue | + | + |
| M25 | Chicken | Tracheal tissue | - | - |
| M26 | Chicken | Tracheal tissue | + | + |
| M27 | Chicken | Tracheal tissue | + | + |
| M28 | Turkey | Tracheal tissue | + | + |
| M29 | Turkey | Tracheal tissue | - (IBV) | - |
| M30 | Turkey | Tracheal tissue | - (IBV) | - |
| M31 | Turkey | Tracheal tissue | + | + |
| M32 | Turkey | Tracheal tissue | - (IBV) | - |
| M33 | Chicken | Pharyngeal swab | - (IBV) | - |
| M34 | Chicken | Tracheal tissue | - (ILT) | - |
| M35 | Chicken | Pharyngeal swab | + | + |
| M36 | Chicken | Pharyngeal swab | + | + |
| M37 | Chicken | Tracheal tissue | - (IBV) | - |
| M38 | Chicken | Tracheal tissue | - (IBV) | - |
| M39 | Chicken | Tracheal tissue | + | - |
| M40 | Chicken | Tracheal tissue | + | + |
| M41 | Chicken | Tracheal tissue | + | - |
| M42 | Chicken | Pharyngeal swab | - (IBV) | - |
| M43 | Chicken | Tracheal tissue | - (ILT) | - |
| M44 | Chicken | Tracheal tissue | - (IBV) | - |
| M45 | Chicken | Tracheal tissue | + | + |
| M46 | Chicken | Tracheal tissue | + | + |
| M47 | Chicken | Tracheal tissue | - (ILT) | - |
| M48 | Chicken | Tracheal tissue | + | - |
| M49 | Chicken | Pharyngeal swab | + | + |
| M50 | Chicken | Tracheal tissue | - (ILT) | - |

Supplemental Table S1-B: Summary of RNA sample panel characteristics based on qRT-PCR screening using the aMPV-SH gene. Discrepancies obtained by RT-LAMP coupled to the DNA nanoprobe detection system (gen F) are indicated in parentheses.

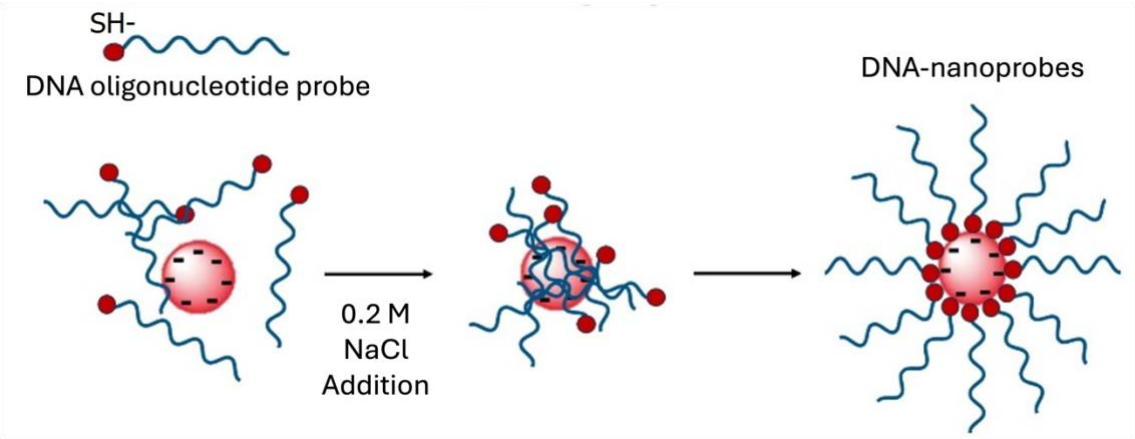
| | Swabs (+) | Swabs (-) | Tissues (+) | Tissues (-) | TOTAL |
|--------------|---------------------------------------|--------------|----------------|----------------|----------------|
| RNA PANEL | 15 | | 35 | | |
| Chickens | 8 | 4 | 12 (8) | 10 | 34 (30) |
| Turkeys | 3 | 0 | 10 | 3 | 16 |
| Subtotal | 11 | 4 | 22 (18) | 13 | |
| TOTAL | 33 (29) positives+17 negatives | | | | 50 |

Supplemental Table S2: Sequences of the primer sets used for aMPV viral RNA amplification by RT-LAMP.

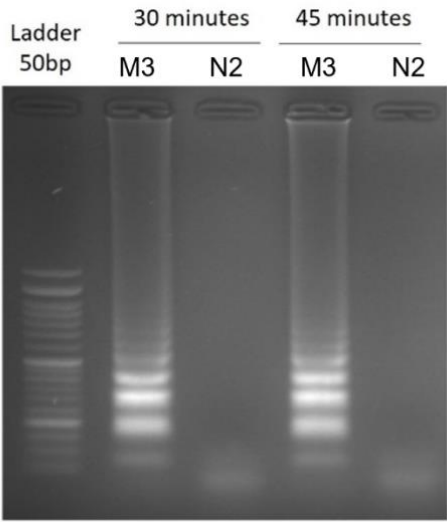
| Target virus | Primer set | Primer name | Sequence (5'-3') |
|--------------|------------|-------------|---|
| aMPV | aMPV-genF | F3-aMPV | GTAATGATGGTCCTAGCCTTA |
| | | B3-aMPV | GCTTTCACCTCCCCTTCT |
| | | FIP-aMPV | CGATTCTCCTTTGTAATCTGATCGGAATTATCACTAACTCAGAATGCCT |
| | | BIP-aMPV | GAAGAGGTTTGTGTTGGGTGCTTTAGCTAAAGCTACGCCG |
| | | LoopF-aMPV | GAAACAGTTCTAAGCTCCTGCA |
| | | LoopB-aMPV | CACCACAGCTGCTGTAACAG |

Supplemental Table S3: DNA oligonucleotide-probe sequences designed for aMPV detection.

| Target virus | Oligonucleotide probe name | Sequence (5'-3') |
|--------------|----------------------------|---|
| aMPV | aMPV-gene-F | TTTTTTTTTTAGGAGCTTAGGCTAGCTACAACTGTTTCT |



Supplemental Fig. S1. Salt aging functionalization of gold nanoparticles (AuNPs) with DNA oligonucleotide probe.



Supplemental Fig. S2. End-point RT-LAMP reaction with the primer set targeting the aMPV sequence gene testing two different reaction times (30 and 45 min) at 65°C. M3 is an isolated RNA from positive aMPV sample and N2 from ILTV RNA used as negative control.