anses
French agency for food, environmental and occupational health & safety
Investigate, evaluate, protect

VIVALDI

European Commission

Project ID : 773422

Detection of avian Influenza A virus by RT-LAMP on VETPOD system

M. FLODROPS, M. CHERBONNEL-PANSART, A. SCHMITZ, FX. BRIAND, C. GUILLOU-CLOAREC, E. NIQUEUX, N. ETERRADOSSI, B. GRASLAND

French Agency for Food, Environment and Occupational Health & Safety (Anses)
Avian and rabbit virology, immunology and parasitology Unit (VIPAC)
National Reference Laboratory for Avian Influenza and Newcastle Disease

PLOUFRAGAN - FRANCE

VIVALDI project

Veterinary Validation of Point of Care Detection Instrument

- Parterns

- Validate new equipment (the VETPOD platform) for rapid on-site detection of zoonotic pathogens (**Avian influenza**, Salmonella and Campylobacter) in industrial food and animal production chains

VETPOD

- VETPOD containing both
  - **Multiplication** of nucleic acids in sample by RT-LAMP (RT-Loop Mediated Isothermal Amplification)
  - **Detection** of turbidity formed by precipitates during the RT-LAMP reaction

VETPOD

- Disposable polymeric **cartridges** (a Lab-on-Chip) performing the biological reactions of pathogen detection.

- The VETPOD system is **generic**, i.e. the cartridges may be loaded with reagents targeting almost any pathogen of choice.

Avian influenza RT-LAMP VETPOD validation method

Reference method
rRT-PCR M-IPC (adapted from Spackman)

1h30 | 1h | 2h | 1h

5 swabs in 1 mL of medium

Pool of 5 swabs

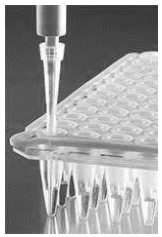
200 µL

RNA extraction

Rneasy Mini Kit (Qiagen)

RNA

Mix preparation rRT-PCR M-IPC



Deposit of mix + samples

Amplification and detection

Data analysis

Gene M detection

200 µL

98° C
10 min

Lysed samples

Samples deposit

Amplification and detection

Reading on VETPOD

Alternative method
RT-LAMP with VETPOD

10 min | 15 min | 55 min

Avian influenza RT-LAMP VETPOD validation plan

- Validation following
  - OIE Terrestrial Manual
  - French standard NF U47-600

I Internal Validation

1. Inclusivity
2. Exclusivity
3. Detection limit of the method
4. Repeatability
5. Intermediate fidelity
6. Diagnostic specificity and sensitivity

II External Validation

1. Recruitment of 5 laboratories
2. Samples preparation
3. Homogeneity and stability testing
4. Send to labs
5. Analyses in labs
6. Analyses of all data

III Validation on the fields

+ and - poultry establishments

I – Internal Validation: Results

1. Inclusivity

28 AIV strains detected
2 strains not detected
H1N1_130045 : Pandemic strain
H6N2_0139/01 : American lineage
Hypothesis : virus quantity or lack of specificity or lysis efficiency

2. Exclusivity

25 non-AIV strains
All strains not detected as expected

3. Detection limit of the method

H5N3_100205b	$10^{4.53}$ EID_{50}/mL
H4N2_100272p	$10^{4.53}$ EID_{50}/mL
H7N9_090034d	$10^{2.46}$ EID_{50}/mL

Same limit of detection as the reference method

4. Repeatability

92.3 % of positive samples were detected with the VETPOD system
100 % of negative samples were not detected

5. Intermediate reproducibility

1 sample (/60) excepted positive was detected negative
98.3% of samples were detected

6. Diagnostic specificity and sensitivity

DSp : 100 %
DSe : 73.68 %

I – Internal Validation: Results

6. Diagnostic sensitivity

- Why were some samples not detected for M gene?
- Why was estimated DSe lower than expected?
  - Inhibition of samples ?
  - Mismatches in primers sequence ?
  - Lysis not efficient ?
    - Comparison of samples: direct thermal lysis vs RNA extraction
    - Test with added sonication step

Test of the 3 hypothesis

I – Internal Validation: Lack of detection hypothesis

6. Diagnostic sensitivity

Hypothesis 1 Inhibition of RT-LAMP	Hypothesis 2 Mismatches	Hypothesis 3 Lysis not efficient
No inhibitor effect	No major shift between primers and M gene sequence	Lysis not fully effective for diagnostic samples Ultrasound seems to improve lysis

II. External Validation : Plan

- 25 samples prepared
  - H5 / H7 / AIV / no-AIV / negative
  - Dilutions (Low/Medium/High)
  - Duplicate samples
- Homogeneity / Stability
- Samples received in the 5 laboratories :
  - 2 Laboratories in France (Labocéa/Labofarm)
  - IZSVe and FLI
  - ANSES (analyses done)

Conclusion

- Fast / effective / easy to implement / inexpensive method

- Adaptation required but high potential to be usable directly on farm

European Commission
Project ID : 773422

VIVALDI

Validation plan of VETPOD system for avian Influenza A viruses

THANK YOU FOR YOUR ATTENTION

Project managed by Marion Flodrops
(Post-doctoral position)