An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

# Optimisation of Serological and Molecular methods for Avian Influenza detection in cattle and results of the 2024 sero-survey

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# One Health EU surveillance project

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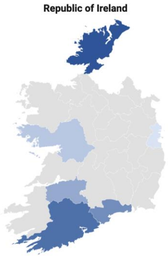
One Health: All Ireland for European Surveillance

**WP2: Enhance surveillance of animal and human Influenza viruses:**

- Wild mammals: foxes, badgers, minks, rodents, feral cats, marine mammals, deer

- Domestic/Farm mammals: cattle, pigs

- Wastewater samples

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# Serological method optimisation bovine serum

Sample selection

| | |
|---|---|
| 1288 | National Cull Cow Scheme from 2023: → cows sent for slaughter (tested BTV, EBL, Brucellosis) from all across Ireland |
| 60 | Longtown (DAFM) farm |
| 10 | Suspect farm samples |
| 6 | 1x Reference serum for H5N1 AI (EURL – IZSVe)<br>5 x Reference sera non-AI pathogens (BVD, IBR, BTV,BCV), Neg |

# Serological methods selections


Screening

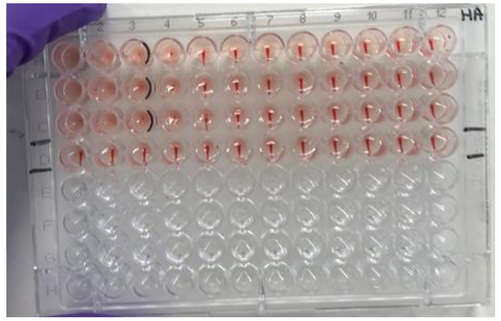
ID.vet ELISA

Influenza A Multispecies

IDEXX ELISA

Influenza A Multispecies

IF positive, Confirmation



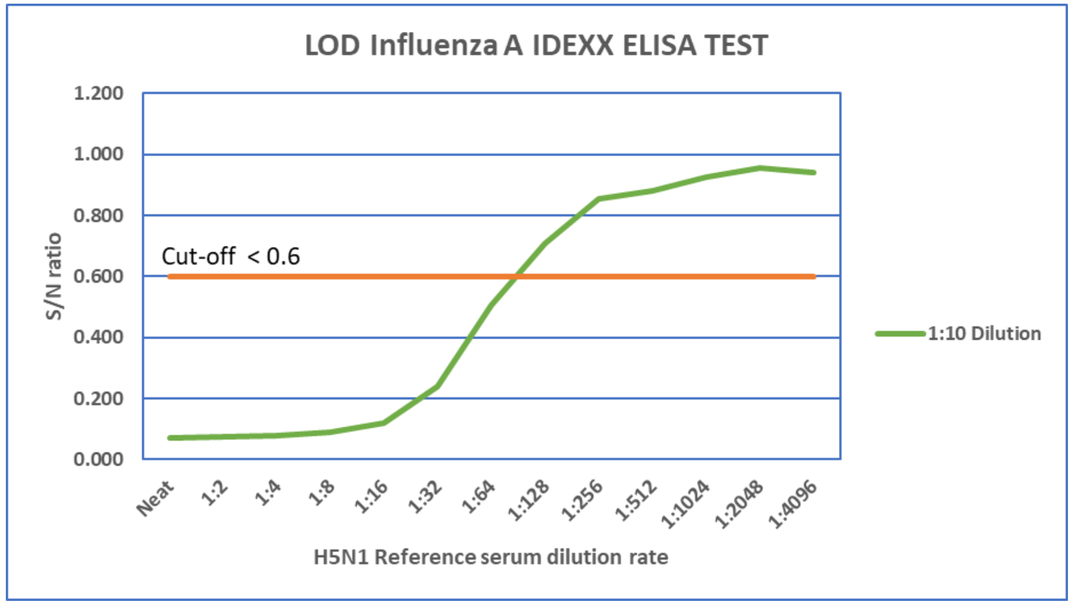
H5 Haemagglutination inhibition (HAI) test in bovine serum

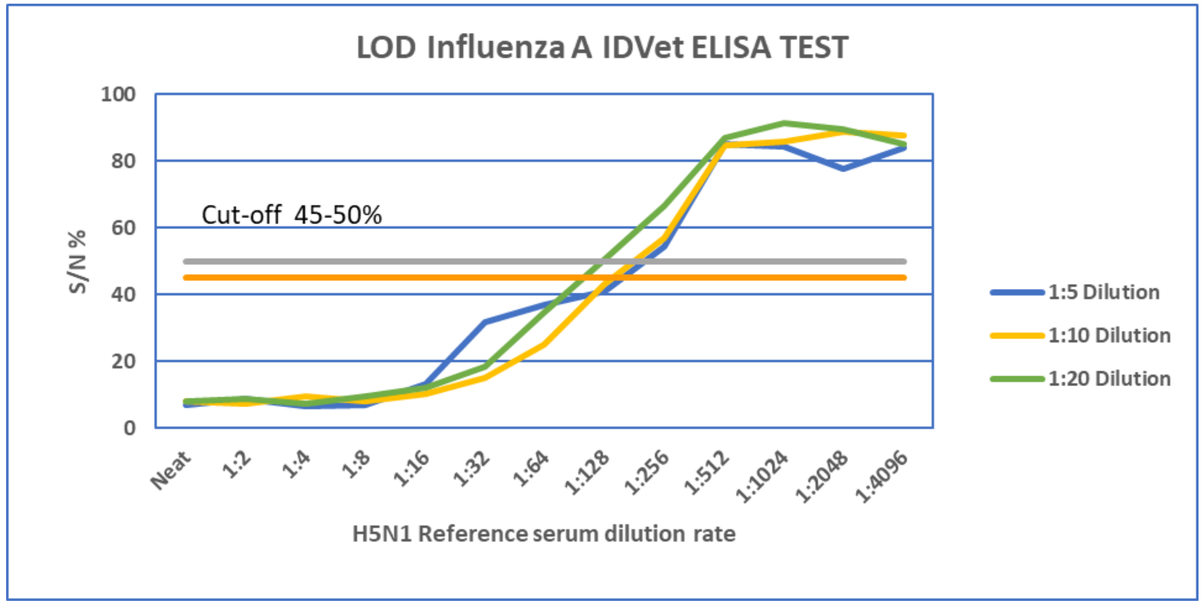
ID.vet H5 Competition 3.0 Multispecies ELISA

HAI also required optimisation with RDE, antigens..

# Laboratory parameters evaluation

- **Limit of Detection (LOD)**
  - Test starting sample dilution:
    - IDEXX: 1:10 as per manufacturer's instructions
    - ID.vet: Tested 1:5, 1:10, 1:20 → Selected 1:10




- LOD Dilution: 1:64 (IDEXX), 1:64 -1:128 (ID.vet) → satisfactory in both kits



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# • Sensitivity and Specificity

| | AI H5 HAI +Pos | AI H5 HAI -Neg | Total |
|---|---|---|---|
| AI IDEXX ELISA +Pos | 0 | 9 | 9 |
| AI IDEXX ELISA -Neg | 0 | 1354 | 1354 |
| Total | 0 | 1363 | **1363** |

**Specificity 99.34%**

| | AI H5 HAI +Pos | AI H5 HAI -Neg | Total |
|---|---|---|---|
| AI IDEXX ELISA +Pos | 1 | 0 | 1 |
| AI IDEXX ELISA-Neg | 0 | 0 | 0 |
| Total | 1 | 0 | **1** |

**Sensitivity 100%**

| | AI H5 HAI +Pos | AI H5 HAI -Neg | Total |
|---|---|---|---|
| AI IDvet ELISA +Pos | 0 | 7 | 7 |
| AI IDvet ELISA -Neg | 0 | 1356 | 1356 |
| Total | 0 | 1363 | **1363** |

**Specificity 99.49%**

| | AI H5 HAI +Pos | AI H5 HAI -Neg | Total |
|---|---|---|---|
| AI IDvet ELISA +Pos | 1 | 0 | 1 |
| AI IDvet ELISA-Neg | 0 | 0 | 0 |
| Total | 1 | 0 | **1** |

**Sensitivity 100%**

| | Sensitivity | Specificity |
|---|---|---|
| IDEXX | 100% | 99.34% |
| ID.vet | 100% | 99.49% |

→ Specificity (>99%) and Sensitivity (100%) in both kits evaluated against ID.vet H5 ELISA and HAI tests.

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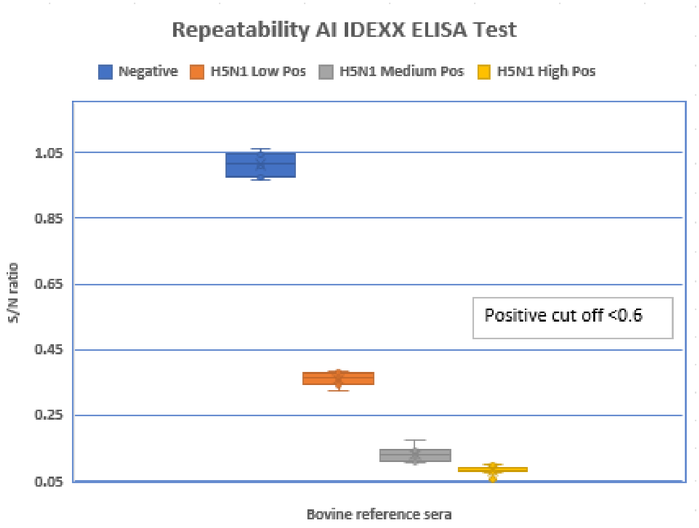
# • Repeatability & Reproducibility

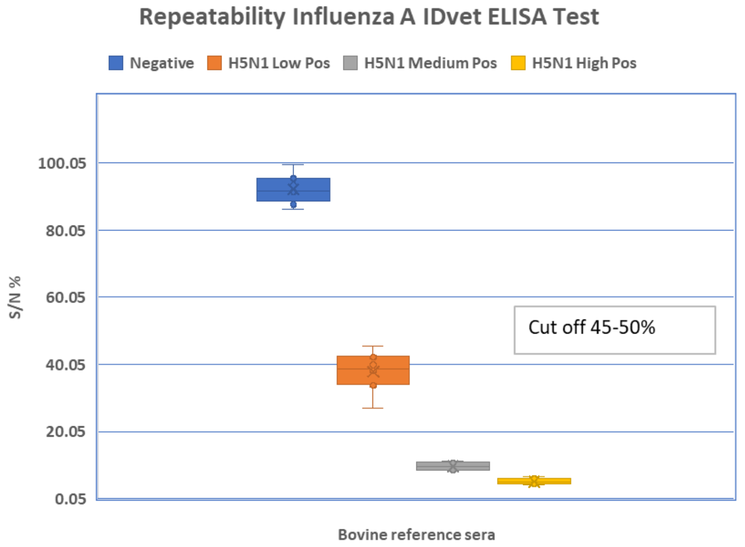
- Bovine reference sera
  - Negative
  - H5N1 low
  - H5N1 Medium
  - H5N1 High

- Repeatability:
  - = conditions
  - = test runs

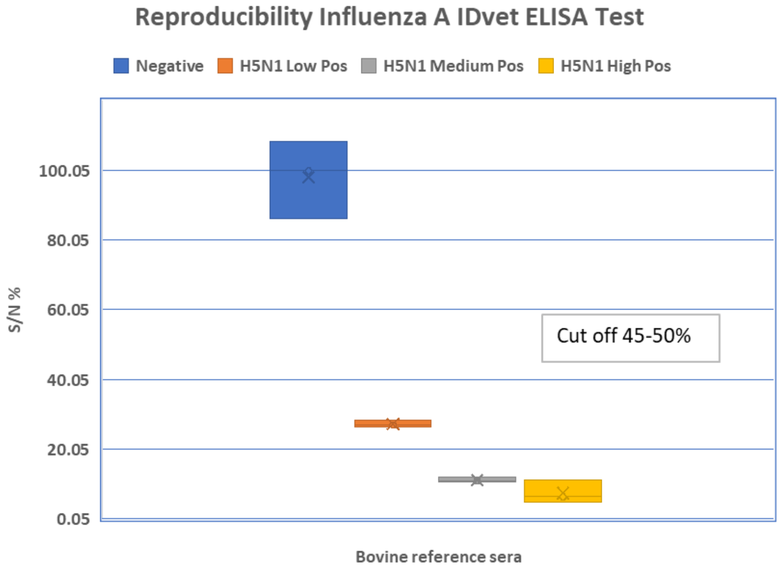
- Reproducibility:
  - ≠ analysts
  - ≠ runs
  - ≠ Kit lot numbers
  - ≠ plate washers

→ Both Kits show <15% CV







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# Results of initial ELISAs comparison

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| | | Influenza A IDEXX ELISA | | Influenza A IDvet ELISA | | |
|---|---|---|---|---|---|---|
| | Sample No. | Negative | Positive | Negative | Doubtful | Positive |
| Reference serum[1] | 6 | 5 | 1 | 5 | 0 | 1 |
| Suspect case[2] | 10 | 10 | 0 | 10 | 0 | 0 |
| Longtown DAFM farm[3] | 60 | 58 | 2 | 60 | 0 | 0 |
| Cull Cow Scheme 2023[4] | 1288 | 1281 | 7 | 1281 | 3 | 4 |
| **Total** | **1364** | **1354** | **10** | **1356** | **3** | **5** |

1.Reference non-AI (BVD, IBR, BTV, BCV) and AI H5N1 EURL IZSVe 2.Suspect case 3.Longtown farm 4.Cull Cow Scheme 2023

- Overall→ Satisfactory results with both kits
- Currently IDEXX is INAB accredited to ISO 17025 for poultry samples in DAFM laboratories→ IDEXX selected for further testing



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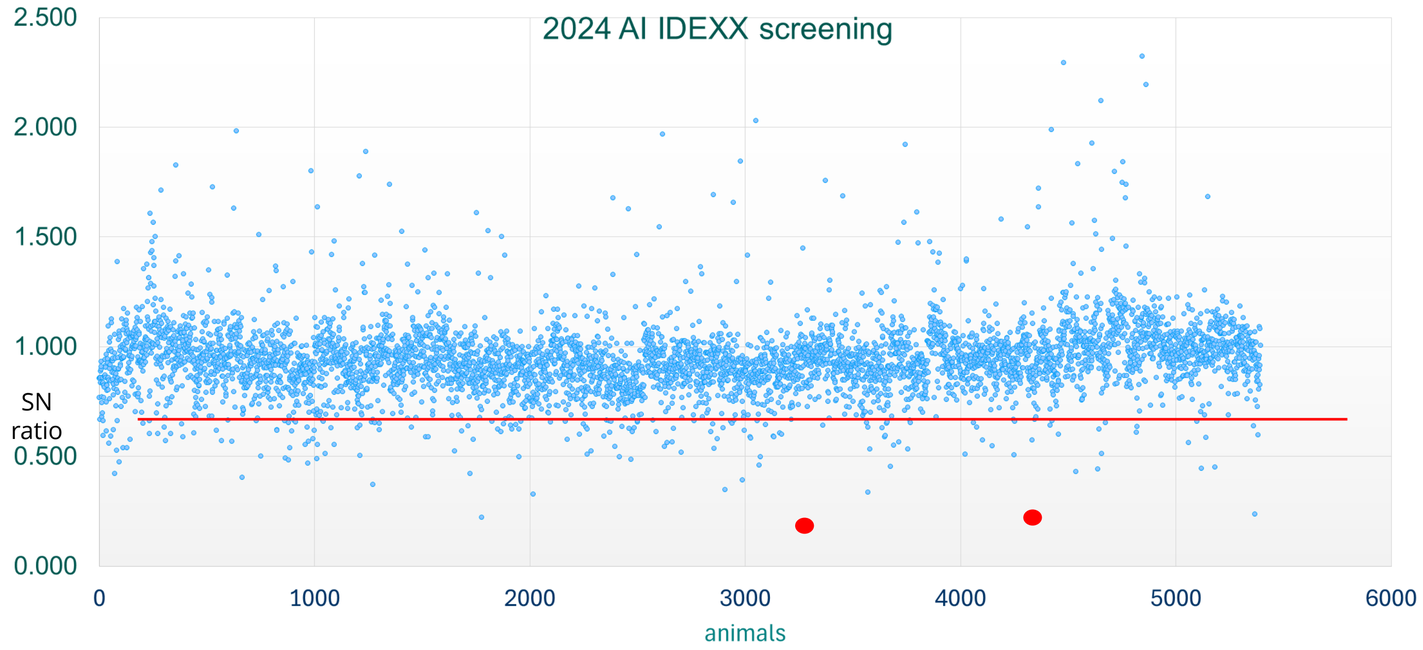
# Sero-surveillance Cull Cow Scheme – 2024




| YEAR 2024 | Screening | | Confirmatory | | | |
|---|---|---|---|---|---|---|
| | AI IDEXX ELISA | | H5 ID.vet ELISA | | H5 HAI | |
| Total tested | Negative | Positive | Negative | Positive | Negative | Positive |
| 5393 | 5325 | 72 | 70 | 2 | 72 | 0 |

Screening:

- IDEXX ELISA → 72 out of 5393 tested positive

Confirmation:

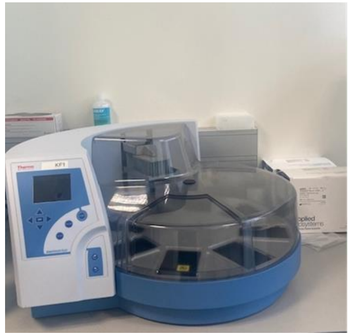
- H5 ID.vet ELISA → 2 out of 72 tested positive
- H5 HAI → all 72 tested negative



**Michaela Loughens, Karely Garcia**

# Molecular method optimisation in milk


- Raw bulk tank milk samples
- Samples diluted 1:3 in Prime Store MTM lysis buffer
- Spiked samples: 3x H5N1 strains 2.3.4.4b _Ireland
- Series of milk dilutions to remove inhibitors and LOD
- Different Incubation conditions
- 2 x methods of RNA extraction evaluated

MagMAX on KingFisherFlex (Thermo Fisher): used for semen samples

MagNA Pure 96 (Roche): used for tissues, swabs, bloods

Matrix Protein AI PCR



**Christina Byrne and Marie Byrnes**

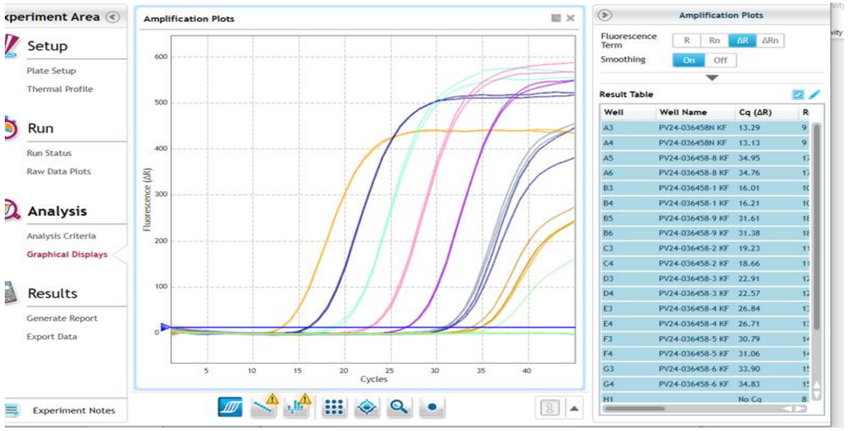
# Results of molecular test optimisation in milk

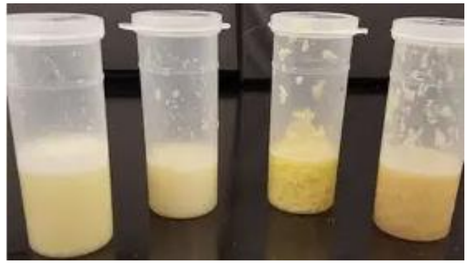
→ KingFisher Flex performed consistently better for the extraction of viral RNA from milk in all experiments

New Testing protocol implemented

Syndromic cases of mastitis and/or reduced milk production of unknown cause tested for AI PCR

Tested 3 x suspect farm cases tested → negative for AI





**Christina Byrne and Marie Byrnes**

# Conclusions

- ✓ Two laboratory methods, one serological and one molecular, optimised in bovine serum and milk, respectively.
- ✓ No evidence of circulating antibodies against Avian Influenza in Irish bovine serum tested so far.
- ✓ No Avian Influenza viral detection by PCR in Irish milk samples tested so far.

# Collaborators

- DAFM :
  - Serology:
    - Michaela Loughens,  Karely Garcia (CVRL-Serology work)
    - Kate O'Keeffe and team (Cork BTL-samples provider, logistics)
    - Damien Barret and team (Ruminant Health - Cull Cow Scheme)
  - Molecular:
    - Christina Byrne and Marie Byrnes (CVRL- Molecular work)
  - Virology Laboratories- DAFM (general support)
  - National Disease Control Center (consultancy)

- External Organisations:
  - EURL- IZSVe (positive controls and protocol guidance)
  - FSAI (consultancy)
  - HSE/HPCS (consultancy)
  - EFSA