GF-TADs
GLOBAL FRAMEWORK FOR THE PROGRESSIVE CONTROL OF TRANSBOUNDARY ANIMAL DISEASES

Food and Agriculture Organization of the United Nations

World Organisation for Animal Health
Founded as OIE

What do we have in the commercially available toolbox now and what are the advantages and disadvantages of excisting systems?

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Diagnostic tools: for which situation, which question?

B. DIAGNOSTIC TECHNIQUES

Table 1. *Test methods available for the diagnosis of avian influenza and their purpose*

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent[1]						
Virus isolation	+	+++	+	+++	+	–
Antigen detection	+	+	+	+	+	–
Real-time RT-PCR	++	+++	++	+++	++	–
Detection of immune response						
AGID	+ (Influenza A)	+ (Influenza A)	++ (Influenza A)	+ (convalescent)	++ (Influenza A)	++ (Influenza A)
HI	+++ (H5 or H7)	++ (H5 or H7)	+++ (H5 or H7)	++ (convalescent)	+++ (H5 or H7)	+++ (H5 or H7)
ELISA	+	+	++	+ (convalescent)	++	++

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
RT-PCR = reverse-transcription polymerase chain reaction; AGID = agar gel immunodiffusion;
HI = haemagglutination inhibition test; ELISA = enzyme-linked immunosorbent assay.

1 A combination of agent identification methods applied on the same clinical sample is recommended.

Potential goals for use of AIV diagnostics?

- Showing freedom of infection
- Diagnostic need: acute infection?
- Check take of vaccine
- Estimation of level of protection induced by vaccination against a certain challenge virus
- Epidemiology, source of infection

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<u>Factors to consider</u>

- Host species?
- Vaccinated vs non-vaccinated flocks
  - Whole virus or DIVA vaccines?
- Epidemic vs endemic
- Multiple HA-subtypes involved?

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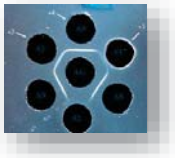
Validated as fit for purpose

Virus detection

- Antigen capture immune assays (point-of-care)
  - Mostly developed/validated for human strains, for influenza A viruses in general
  - Varying sensitivity, often 3 to 4 $\log_{10}$ less sensitive compared to VI
  - Recommended for strongly positive samples only (like HP, clinically affected or dead birds, flock level)

- RT-PCR
  - Conserved gene (usually M), HA subtype specific (e.g., H5), N-subtypes
  - Should be properly validated using clinical material to demonstrate tests as 'fit for purpose'
  - Highly sensitive (high CT might not mean an active infection anymore)
  - Importance of continuously monitoring primers and probes (combination of M and Np might be best)
  - All species

Antibody detection



- Agar gel immune diffusion test
  - Influenza A specific (antibodies against nucleoprotein and matrix antigens)
  - Precipitating antibodies, suitable for chicken and turkeys, less reliable in other species
  - Best in detecting acute infections, flock diagnosis
- Hemagglutination inhibition test
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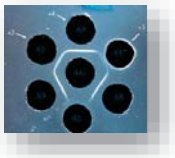

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  - Subtype specific
  - All species
  - Non-chicken sera might need absorption with chicken red blood cells before testing to prevent nonspecific agglutination.
  - Potential nonspecific inhibition of agglutination caused by steric inhibition when the tested serum contains antibodies against the same N subtype as the H antigen used in the HI test.
    - Use of two antigens for each haemagglutinin subtype with heterologous neuraminidase (i.e. H5N1 and H5N6)
      - No H5N2 antigen in H9N2 endemic areas
    - Alternatively, the H antigen used can be recombinant or purified H protein that lacks N protein
  - Strong correlation between HI titres and level of protection when homologous antigen is used
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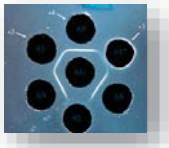

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- ELISA
  - Antigen: whole virus, nucleoprotein or HA H5 (some other proteins less immunogenic)
  - Indirect (chicken/turkey conjugate), blocking (all species)
  - ELISA titres have poor correlation with protection (all kind of antibodies, not only 'protective' antibodies)

Current H5 vaccines and DIVA options

- Vaccines
  - Inactivated complete virus
  - Subunit vaccine,
  - Live vectored vaccines (HVT, Pox, others)
  - mRNA, DNA, ……

Only antibody response against insert (e.g.) H5
Not against other proteins (M, Np, …..)

Find a suitable DIVA combination

- Tests
  - RT-PCR, virus isolation, staining, on-site, ELISA (general), ELISA (specific proteins), genotype specific ELISA, HI-test, AGPT

- Field situation, non-H5 strains/vaccinations?

Check for freedom of subtype H5 virus (infection), success of vaccination (commercially available tests/antigens)

Field situation	AGID	HI H5	ELISA			Antigen	RT-PCR	
			Whole virus	Np	H5		M	H5
No AIV vac, no other subtype chall	+	++	++	++	++	+	++	++
No H5 vac, other subtype vac/chall	-	++ (N!)	-	-	++	+	++	++
H5 vac using whole virus	-	-	-	-	-	+	++	++
H5 DIVA vac, no other subtype vac/chall	+?	-	-	++	-	+	++	++
H5 DIVA vac + other subtype vac/chall	-	-	-	-	-	+	++	++
Level of take of H5 vaccine	±	++ (hom vaccine)	++		++	-	-	-
Estimation H5 protection level	-	++ (hom field)	-	-	±	-	-	-

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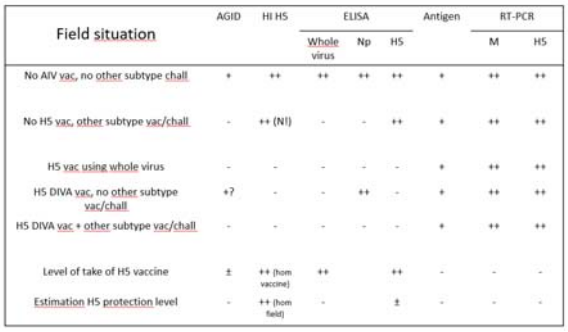
Take home message



- For any situation, DIVA testing using commercially available tests is possible
- However, the number of options varies depending on the field situation

Thank you for your attention