Fit for purpose diagnostic tools for monitoring and surveillance in vaccinated populations

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Vaccination for Avian Influenza

- Vaccination protects from disease but not infection (no sterilizing immunity)
- Vaccination can reduce transmission between vaccinated flocks
- Primary protection from antibody to hemagglutinin protein
- Limited protection from antibody to neuraminidase and matrix 2 (M2) protein
- No protection from antibodies to internal proteins (nucleoprotein, matrix 1, and NS1)
- Cell mediated immunity of additional value
- No cross protection between subtypes-i.e. H5 vaccines only protect against H5 viruses

Antibody Response After Vaccination

- Vaccination that induces high levels of neutralizing antibody will greatly reduce but not prevent infection
- Virus replication and virus shed will be reduced in well vaccinated birds lowering virus antigen available to stimulate immune response
- Vaccinated birds may have reduced or delayed antibody response after infection to the DIVA antigens

DIVA

- **D**ifferentiate **I**nfected from **V**accinated **A**nimals
- **DIVA** principle primary application is to assure trading partners that livestock have not been exposed to infectious virus i.e. **differentiate vaccinated only and vaccinated and then infected poultry**
- Inexpensive, reliable, and high throughput differential serologic test needed to make DIVA surveillance testing viable
- qRT-PCR testing will also play a role in testing
- Sentinel birds and Flock Status
- For countries that do not export poultry, DIVA vaccination probably not a major priority

Serologic Diagnostic Tests for Avian Influenza

- Tests to identify **any** type A influenza virus
  - Agar gel immunodiffusion test (AGID) targeted matrix or nuceoprotein
  - Commercial ELISAs' targeted to nucleoprotein
- Subtype specific
  - Hemagglutination inhibition (HI) targeted to hemagglutinin protein and is **subtype specific**
  - Neuraminidase inhibition (ELLA) targeted to neuraminidase enzyme and is **subtype specific**
  - Neuraminidase subtype ELISA tests are commercially available for N1 and N2

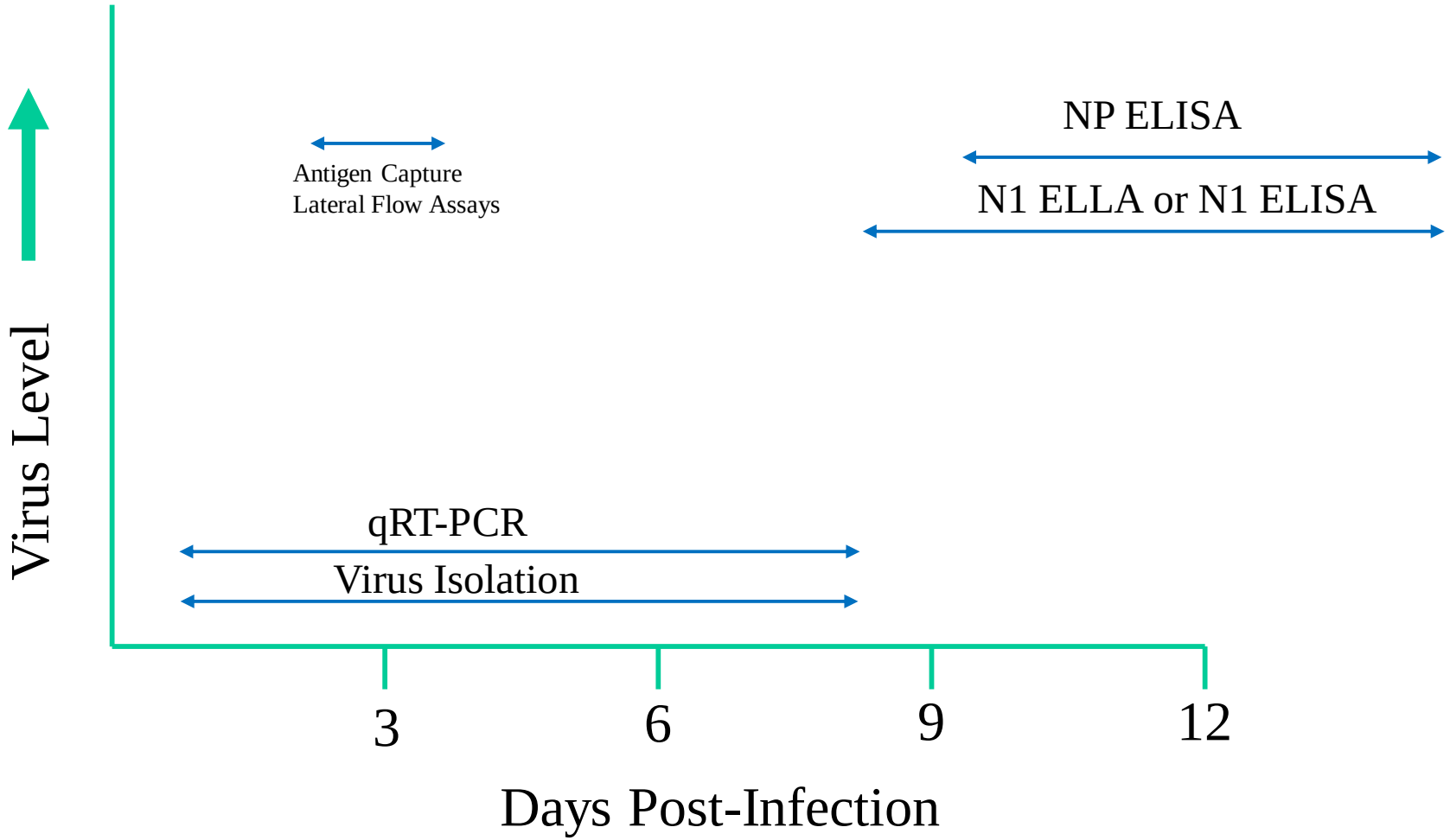
Monitoring Flock Response to Vaccination

- Understanding the number of birds that immunologically respond to vaccination and the level of response is predictive of protection
- Hemagglutination inhibition tests remains the gold standard for evaluating level of immune response
  - Antigenic variation is critical in this response
  - Using currently circulating field antigen provides more predictive results for protection
  - HI tests are more variable between laboratories and are difficult to perform at higher scale
- HA specific ELISA tests or NP ELISA for whole virus vaccines can provide some evidence of percentage of birds that responded to vaccine

Differentiation of Infected from Vaccinated Animals (DIVA) Serologic Approaches

- Detection of NP antibodies to the field virus
  - Can only be used with subunit (HA only) vaccines
  - Commercial NP ELISA tests readily available
- Detection of antibodies to the NA protein of the field virus
  - Requires vaccination heterologous NA vaccine
  - ELLA= Enzyme linked lectin assay (not commercially available)
  - ELISA neuraminidase antibody test (available in Europe)
- NS1, M2e, HA2 subunit vaccines proposed serologic DIVA strategies remain at experimental stage

Avian Influenza Diagnostic Tests-Range of Detection in Subunit Vaccinated/Challenged Flocks

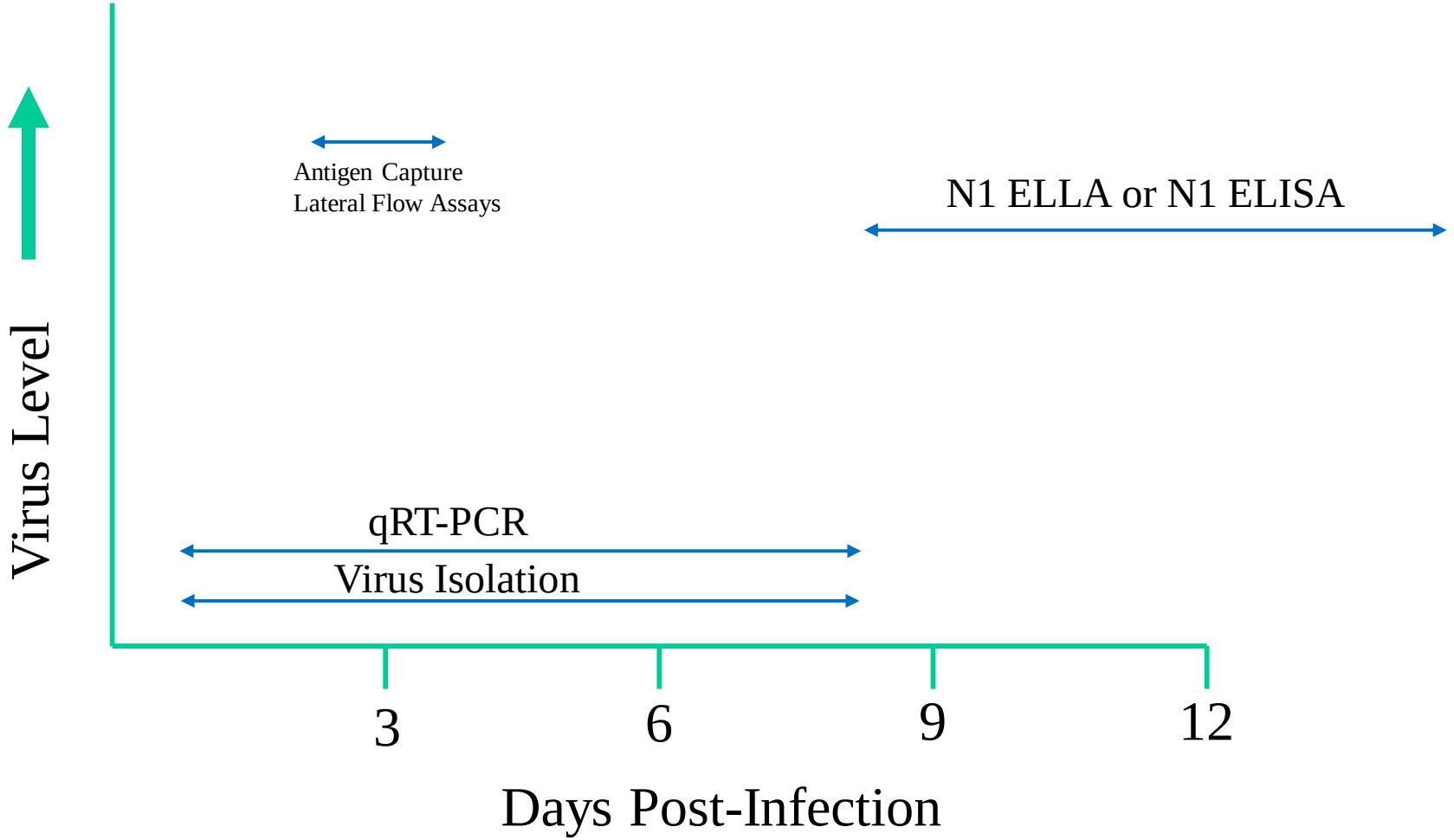


DIVA test: percent detection of infection induced NA or NP antibody with rHVT vaccines

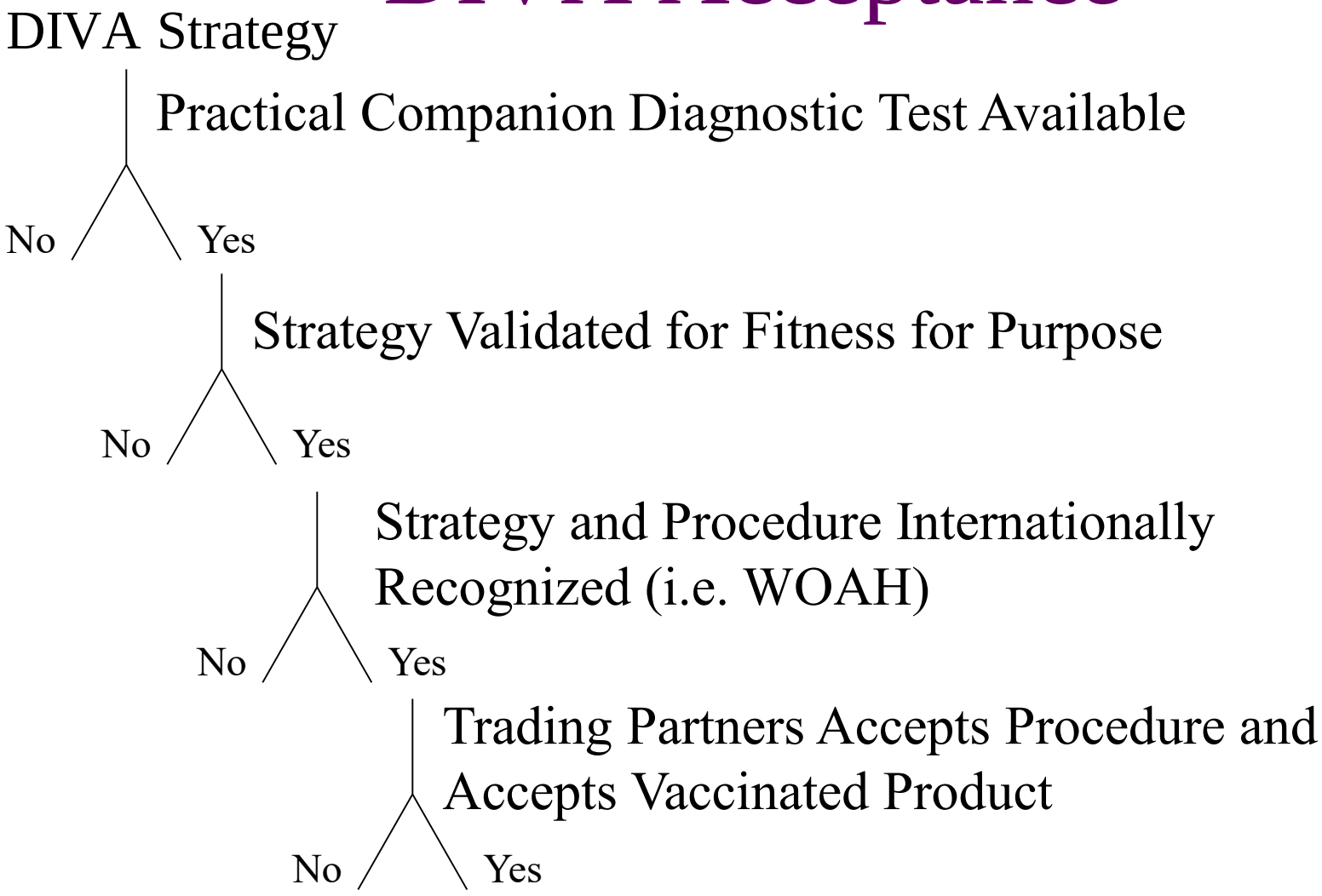
Vaccine	Breed	ELLA-NI			ELISA (NP)		
		7 DPC	10 DPC	14 DPC	7 DPC	10 DPC	14 DPC
COBRA -HVT	White leghorn	100	100	100	5	25	25
	Broiler	100	100	100	10	20	10
2.2-HVT	White leghorn	100	100	100	16.7	27.8	27.8
	Broiler	100	100	100	11.1	75	55.5

- NP antibody ELISAs were not optimized for DIVA
- N1 antibody test: concerns with NA reassortants

AI Diagnostic Tests-Range of Detection in Whole Virus Heterologous NA Vaccinated/Challenged Flocks



DIVA Acceptance



Sentinel Birds

- Maintain unvaccinated birds throughout house or farm
- Birds are individually marked, but otherwise treated like vaccinated birds
- Birds are sampled routinely by serology or virology methods
- Classic method of surveillance to show whether flock was exposed to the agent in question

Flock Status

- Monitor flock on daily basis for key measures of health
  - Daily mortality
  - Water consumption
  - Feed consumption
  - Egg production
- Virologic testing (qRT-PCR) when any factor increases beyond normal
  - Daily mortality (may be only a small increase)
  - Sick birds

qRT-PCR for DIVA Surveillance

- qRT-PCR is well established sensitive and specific test for avian influenza
- Remains primary option to test birds or product for virus at the time of processing
- Costs and logistics of increasing amount of testing remains a hurdle

Issues with Current DIVA Strategies

- Validation or fitness for purpose not completed for any method for DIVA surveillance
  - No internationally recognized companion DIVA tests are available
  - Poultry with high HI antibody levels may not seroconvert well to challenge
    - Will this require surveillance on a larger number of samples per flock
  - Because immune response is not uniform, will the standard be zero tolerance of antibody
  - Transparent surveillance and publication of methods needed for broader acceptance