

Different WN serological methods for human, bird, chicken and horse

- **ELISA:**
 - Indirect ELISA for IgG
 - Capture ELISA for IgM
- **Plaque Reduction neutralisation test (PRNT):**

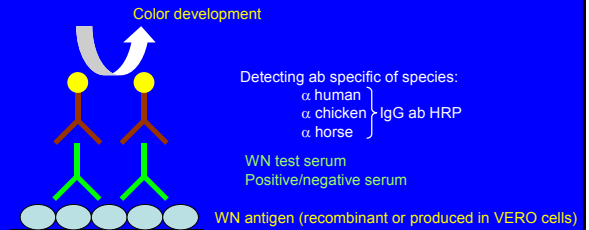
Standard PRNT
Micro PRNT



Complementarity between methods
Different purposes

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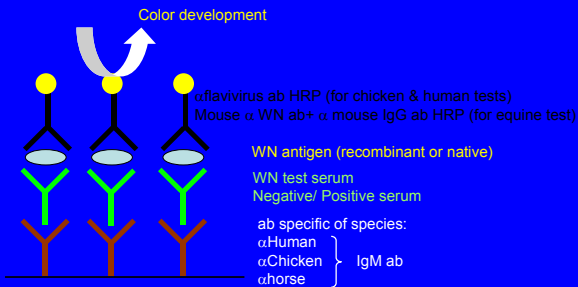
Indirect ELISA for WN IgG in human chicken & horse



One indirect ELISA for IgG in wild birds: use of anti-wild bird IgG ab HRP
Problem of high background (positive serum reacting with uninfected antigen)

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WN IgM capture ELISA in human, chicken & horse



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Measure of antibody titer for IgG and IgM ELISA:

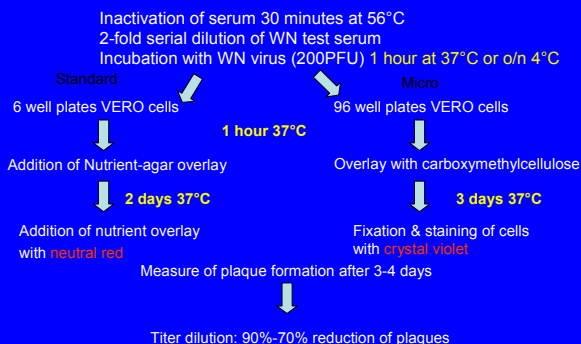
Cut-off value: OD of negative controls+3 times SD
Positive serum when $OD_{\text{measured}} \geq \text{Cut-off value}$

Positive to negative ratio

$P/N = OD_{\text{test serum}} / OD_{\text{negative serum reacting with viral antigen}}$
P/N positive control ≥ 2
Positive serum when $P/N \geq 2$

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Standard & Micro Plaque Reduction Neutralisation Test (PRNT)



Advantages and disadvantages of WN ELISA

ELISA

Easy
Rapid
Reliable method

Need a secondary ab specific of species

IgM capture ELISA:

- Detection of early infection (3 days-2 months)
- Limited to surveillance conditions
- Minimal cross reactivity with SLEV
- No detection of free ranging birds

IgG indirect ELISA:

- Long term detection of ab
- Possibility to detect WN antibodies from wild birds
- Some cross reactivity with other flavivirus

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Advantages and disadvantages of PRNT

PRNT

Technical complexity

Time consuming

Expensive

Necessity to have live virus (L3 lab security level)

- Confirmation of the specificity of WN + serum
- Use of PRNT for serum from species when secondary conjugate ab not available
- Micro-PRNT detects both IgM and IgG compare to standard PRNT