

Epitope-blocking ELISAs



- **Important to distinguish between WNV & SLEV**

- Overlapping geographic distributions
- Closely related antigenically
- Maintained in similar transmission cycles

- **Disadvantages of other serologic techniques**

- PRNT: laborious, expensive, require live virus
- HI assay: laborious & not WNV-specific
- Direct ELISA: a different 2° Ab is required for each species, not WNV-specific

Epitope-blocking ELISAs

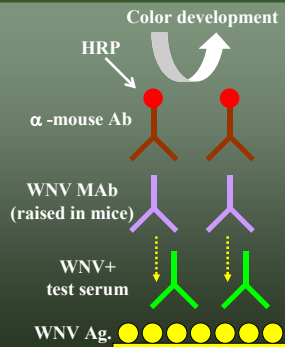


Developed by Blitvich et al (Fort Collins university, Colorado)

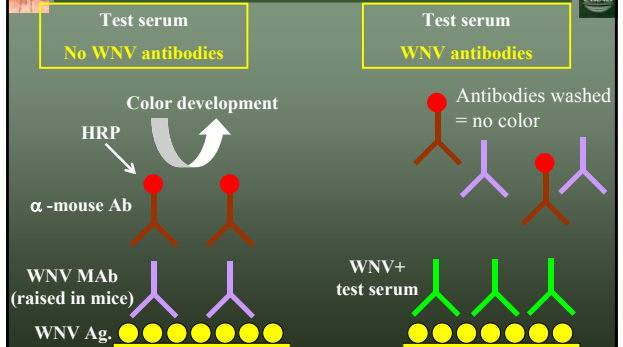
- ✓ Epitope-blocking ELISA for detection of West Nile virus antibodies in domestic mammals, JCM, 2003, 41,2676-9
- ✓ Epitope-blocking ELISA for the detection of serum antibodies to West Nile virus in multiple avian species, JCM, 2003, 41,1041-7

Adapted in Guadeloupe in collaboration with B Blitvich

Epitope-blocking ELISAs



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Epitope blocking ELISA Protocol



Coating plate NUNC Maxisorp WNV antigen in coating buffer 1/4000 O.N. at 4°C + 1h at 37°C
Wash 4X (PBS 1x, 0.1% tween 20)
Blocking (PBS 1x, 0.1% tween 20, 0.2% BSA), 37°C, 45 min
Wash 1x
Serum 50 µl serum diluted 1/10 in blocking buffer, 37°C, 2h
Wash 4x
Monoclonal antibody 50 µl of Mab anti WNV 3.1112G 1/4000 blocking buffer, 37°C 1h + 4°C 1h
Wash 4x
Conjugated antibody 50 µl HRP rabbit anti mouse IgG 1/500 in blocking buffer, 37°C, 1h
Wash 4x
 200 µl of TMB working solution, 30 min at 37°C
 After 30 minutes, add 100 µl H2SO4 2N – read OD at 450nm

Epitope blocking ELISA Protocol



	external wells								
0 Ag	Ech. 1	Ech. 4	Ech. 7	Ech. 10	Ech. 13	Ech. 16	Ech. 19	Ech. 22	Ech. 25
0 Ag	Ech. 1	Ech. 4	Ech. 7	Ech. 10	Ech. 13	Ech. 16	Ech. 19	Ech. 22	Ech. 25
T-	Ech. 2	Ech. 5	Ech. 8	Ech. 11	Ech. 14	Ech. 17	Ech. 20	Ech. 23	Ech. 26
T-	Ech. 2	Ech. 5	Ech. 8	Ech. 11	Ech. 14	Ech. 17	Ech. 20	Ech. 23	Ech. 26
T+	Ech. 3	Ech. 6	Ech. 9	Ech. 12	Ech. 15	Ech. 18	Ech. 21	Ech. 24	T+
T+	Ech. 3	Ech. 6	Ech. 9	Ech. 12	Ech. 15	Ech. 18	Ech. 21	Ech. 24	T+

Calculation of % inhibition of MAB binding

$$\% \text{ inhibition} = 100 - \frac{(TS - B)}{(CS - B)} \times 100$$

TS = OD of test sera
 CS = OD of control sera (from uninfected animal of same/similar sp)
 B = background OD

≥30% indicates the presence of flavivirus antibodies

Epitope blocking ELISA Protocol



- VALIDATION

- OD Blank (0 Ag) < 0.15
- 0.30 < OD T- < 0.9
- % inhibition T+ > 75%

- INTERPRETATION

- The ability of the test sera to block the binding of the MAbs to WNV antigen was compared to the blocking ability of negative serum. Data were expressed as relative percentages and an inhibition value >30% was considered to indicate the presence of viral antibodies.
- Inhibition of the test sample = $100 - 100 * (\text{OD of the test sample} - \text{OD of the blank}) / (\text{OD of negative sample of the same specie} - \text{OD of the blank})$

- BUFFER and SOLUTION

- REAGENTS

- LABORATORY EQUIPMENT

- LABORATORY CONSUMABLES