

West Nile Virus Serology

Is ELISA challenged by the
arbovirus IFA test and new
technologies?

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Current Standards for Serological Detection

- ⌘ IgM Antibody Capture ELISA (MAC-ELISA) with CDC reagents
- ⌘ IgG indirect (sandwich) ELISA with CDC reagents
- ⌘ Verification by cross-species plaque neutralization testing on paired specimens against likely viruses

Comparison of Antibody Levels in Serum and CSF

	Serum (g/L)	C.S.F (g/L)	Serum/ CSF
IgG	8-15	0.01- 0.014	1000
IgA	0.9-3.2	0.001- 0.003	1000
IgM	0.45-1.5	0.0001- 0.0012	1666

Why IgM antibody capture?

- ⌘ If indirect (sandwich) ELISA technique were used for IgM, the **IgG would out compete** for available antigen binding sites on the WN Envelope antigen, and the small amount (10-100X less) of IgM would not be detected!
- ⌘ Reaction (binding) kinetics of sandwich ELISA favor IgG

MRL (Focus Technologies) Arbovirus IFA Test

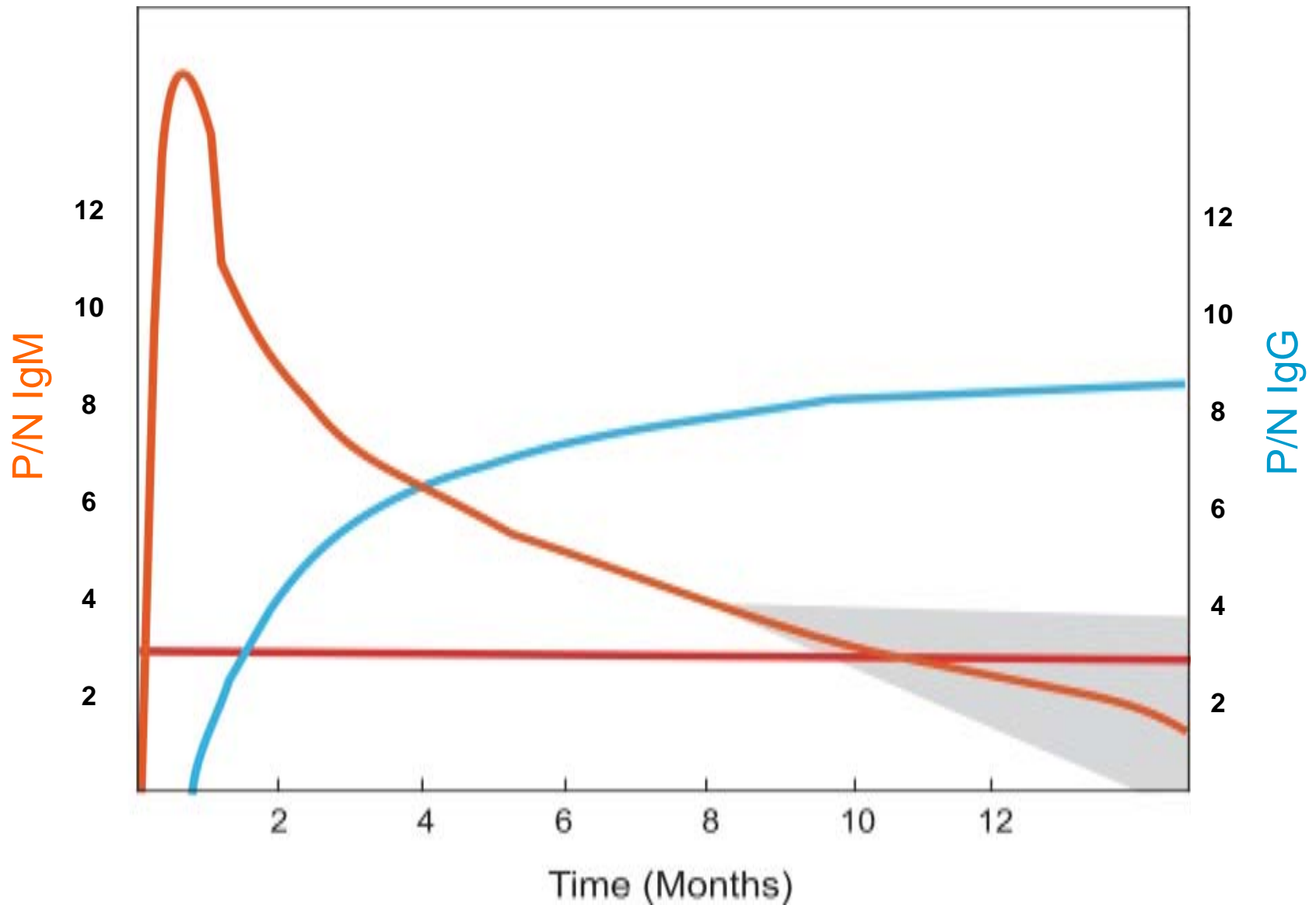
- ⌘ Four different North American arboviruses are present in Vero cells dotted and fixed on each well of the slide
 - ☒ Eastern Equine Encephalitis
 - ☒ Western Equine Encephalitis
 - ☒ La Cross Encephalitis
 - ☒ St. Louis Encephalitis
- ⌘ Screening for Alpha, Flavi- and Bunya viruses

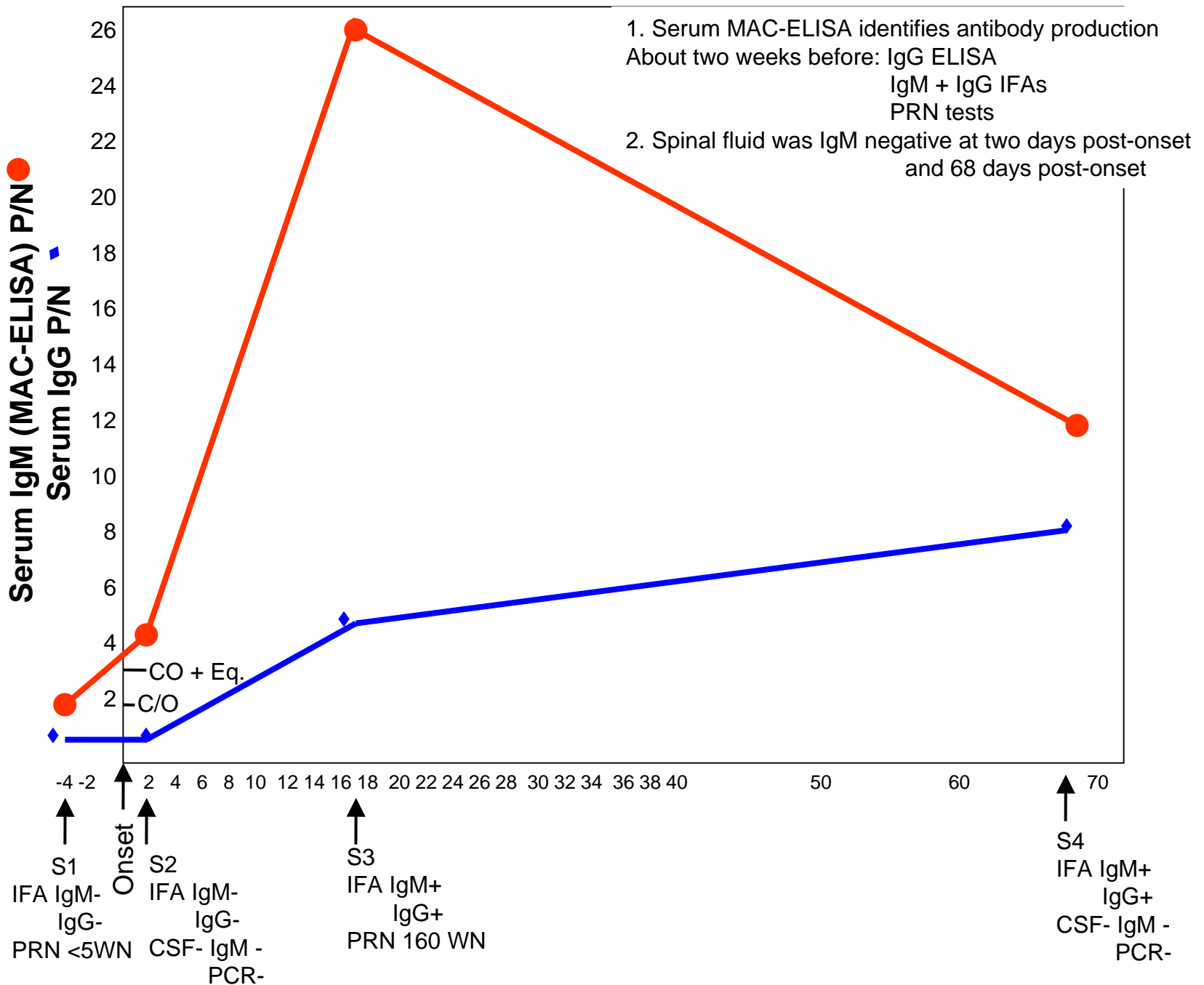
IFA Requirements

- ⌘ Trained observer interprets pattern of fluorescence
- ⌘ Recognizes autoantibodies and non-specific binding
- ⌘ Recognizes correct proportion of stained infected cells

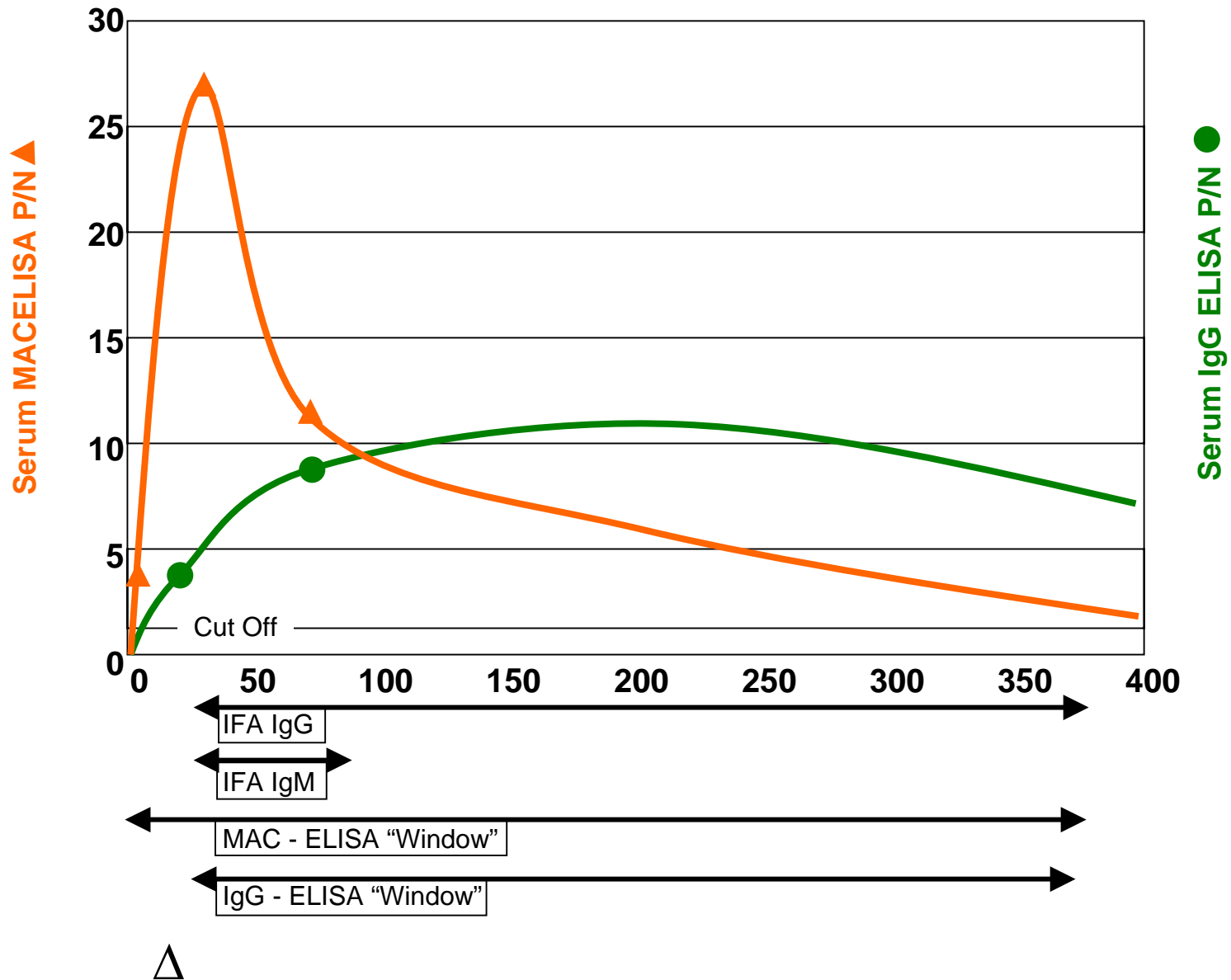


Antibody Response to WNV





Detection Windows of Opportunity



1999 WNV Outbreak Sera Comparison: IFA to ELISAs and PRN

SLE results	Reactive n (%)	Cross- Reactive n (%)	Non- reactive n (%)	Non- specific n (%)
Arb IgM IFA	3 (10.7)	6 (21.4)	17 (60.7)	2 (7.1)
Arbo IgG	26 (92.9)		2 (7.1)	

Review of 1999 IFA Results

- ⌘ Three sera positive by IFA IgM test were collected 10 to 15 days post onset of symptoms
- ⌘ Sera reactive in the MAC-ELISA were collected from 1 to 30 days post onset
- ⌘ Good sensitivity of the IgG IFA compared to IgG ELISA
- ⌘ Possibly a 10 to 15 day time advantage of MAC-ELISA? Broader “window....

2001 IFA IgG Results

⌘ All sera (51) reactive in the IgG ELISA
were reactive in the IgG IFA test to SLE

⌘ 6 sera reactive in the IFA test, non-
reactive in the IgG ELISA

☒ 1 patient WNV

☒ 5 patients no follow-up

2001 Arbovirus Antibody Comparison: IFA to ELISAs and PRN

⌘ 45 encephalitis patients (82 specimens) with at least one positive result (ELISA, IFA)

⌘ 30 patients with IgG ELISA +ve or Eq

⊠ 9 (30%) WNV

⊠ 4 (13.3%) Flavivirus

⊠ 3 (10%) Dengue

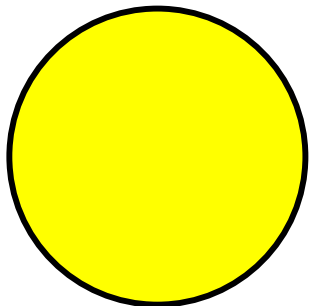
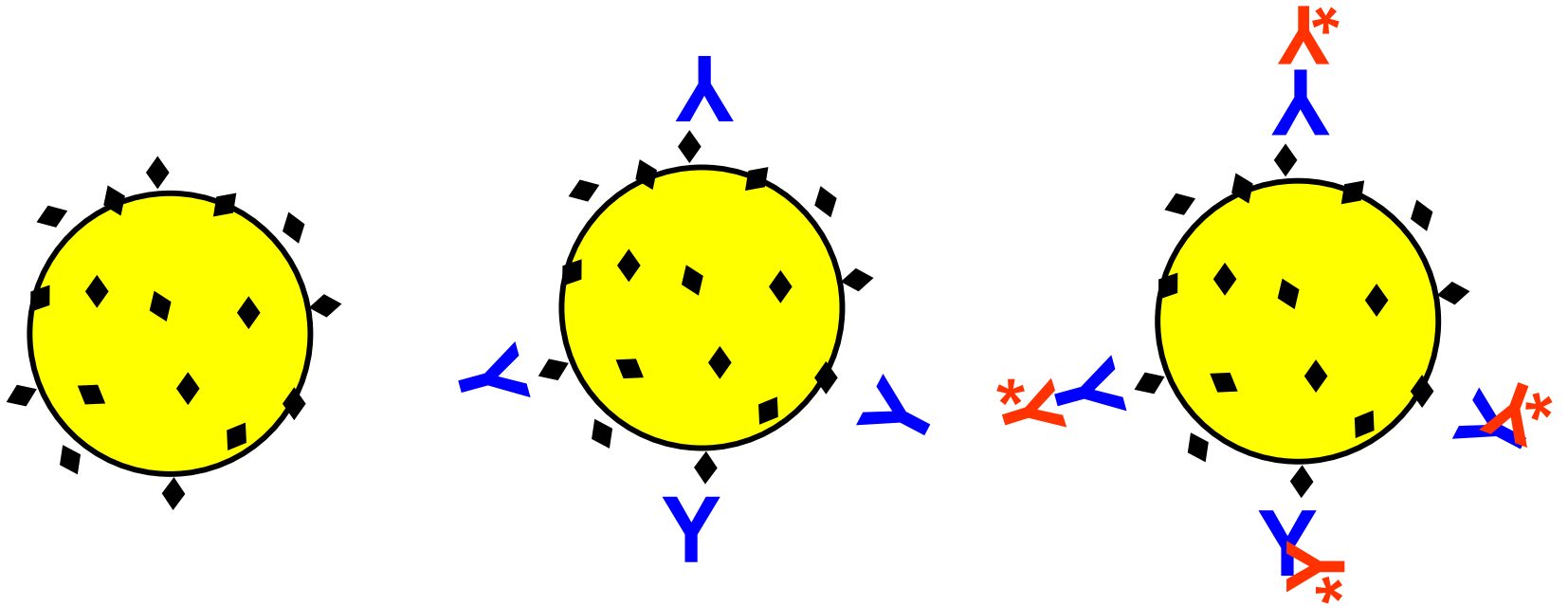
⊠ 1 (3.3%) Nonreactive by PRN

⊠ 13 (43.3%) no follow up, no PRN

2001 IFA IgM Results

- ⌘ All patients with a positive or equivocal MAC ELISA tested on IgM IFA
- ⌘ All patients (8) with reactive IgG IFAs other than SLE were tested on IgM IFA
- ⌘ **One patient only reactive IgM to SLE in the IFA test (titer 2048, P/N 26), d 17, d 68**
- ⌘ Mean P/N 17.78 (7.21-26.2) first sera
- ⌘ Mean P/N 15.53 (8.35-19.5) follow up sera

Suspension Array Technology Sequence of Events



Fluorescent Bead -
Yellow

◆ Antigen - Black

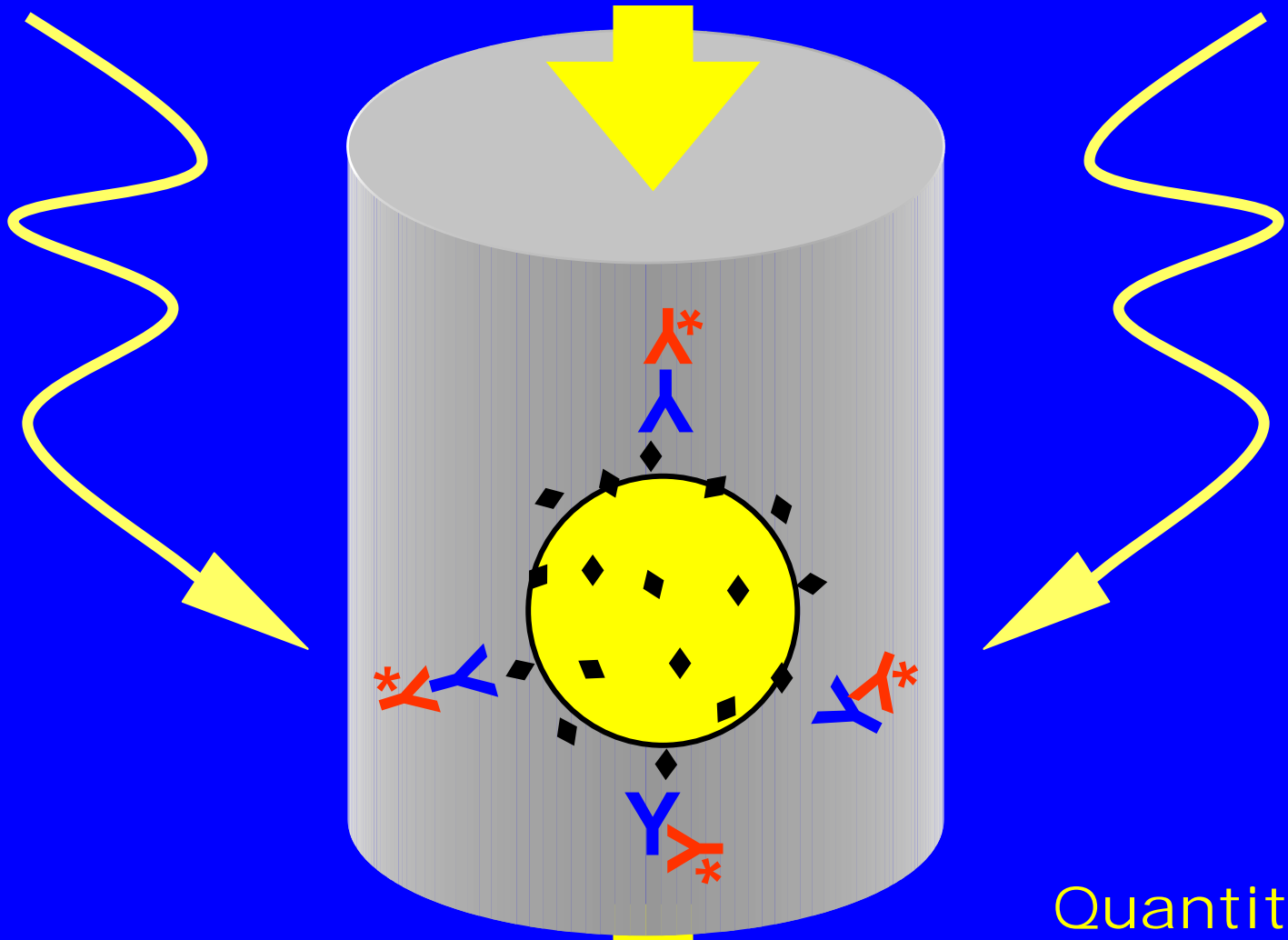
Y Patient's Antibody - Blue

*Y PE Labeled Anti Antibody - Red

Laser 1

Flow Cell

Laser 2



Bead
Identification

Quantitative
Measure of
Extrinsic
Fluorescence

Luminex Analyzer



Data Analysis of Multiplex Assay



Reaction in Multiwell Filter Plates



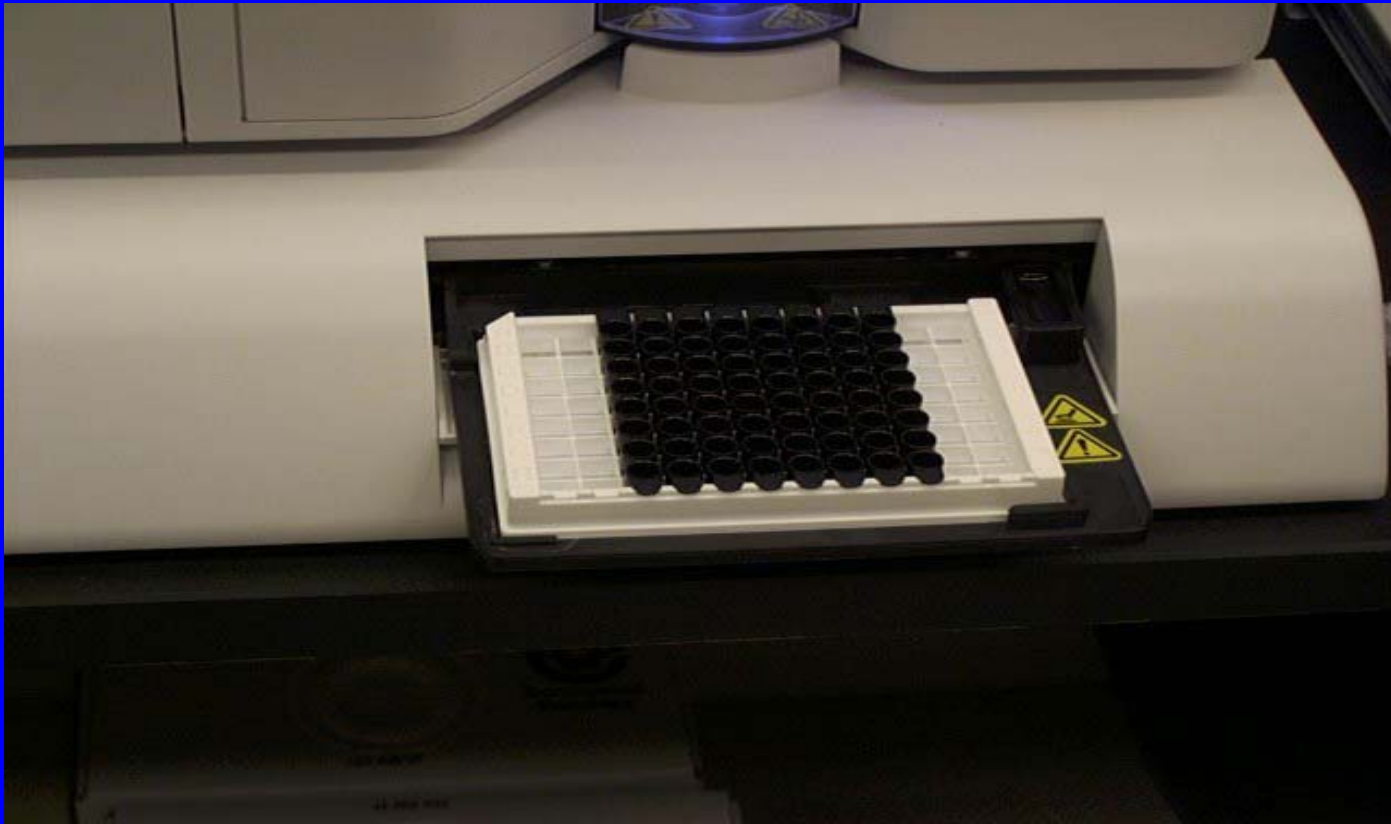
Pump, Filter Plate, Sonicator and Vortex



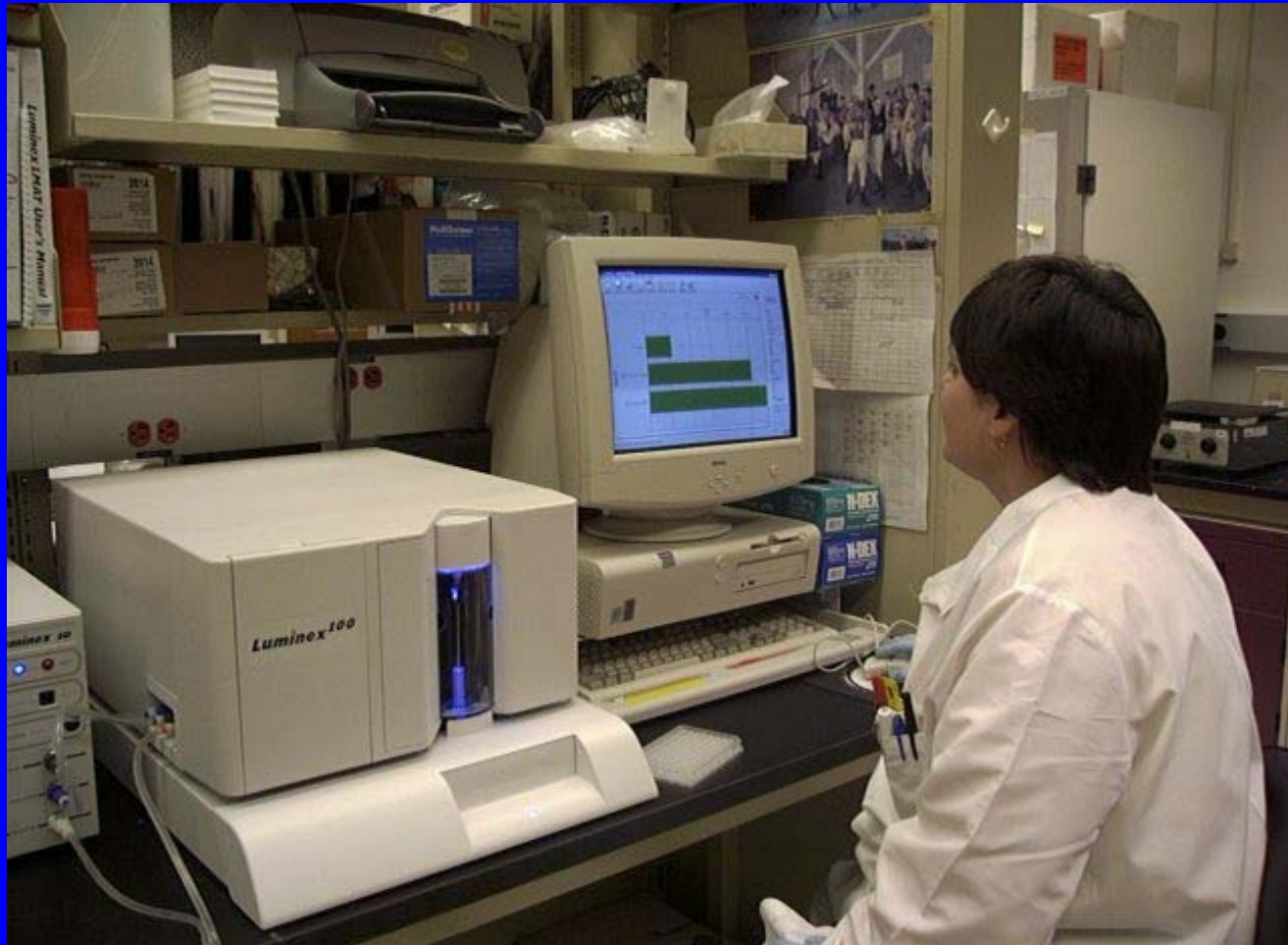
Partial Plate in Use for Small Assays



Black Strip Wells Carry Beads into Analyzer



Data Review by Technologist



Acknowledgements

⌘ CDC ELC WNV grant

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