Duplex Microsphere Immunoassay for Detection of IgM to WN and SLE Viruses

Further Developments and Validation

SAFER • HEALTHIER • PEOPLE

Objective of the MIA

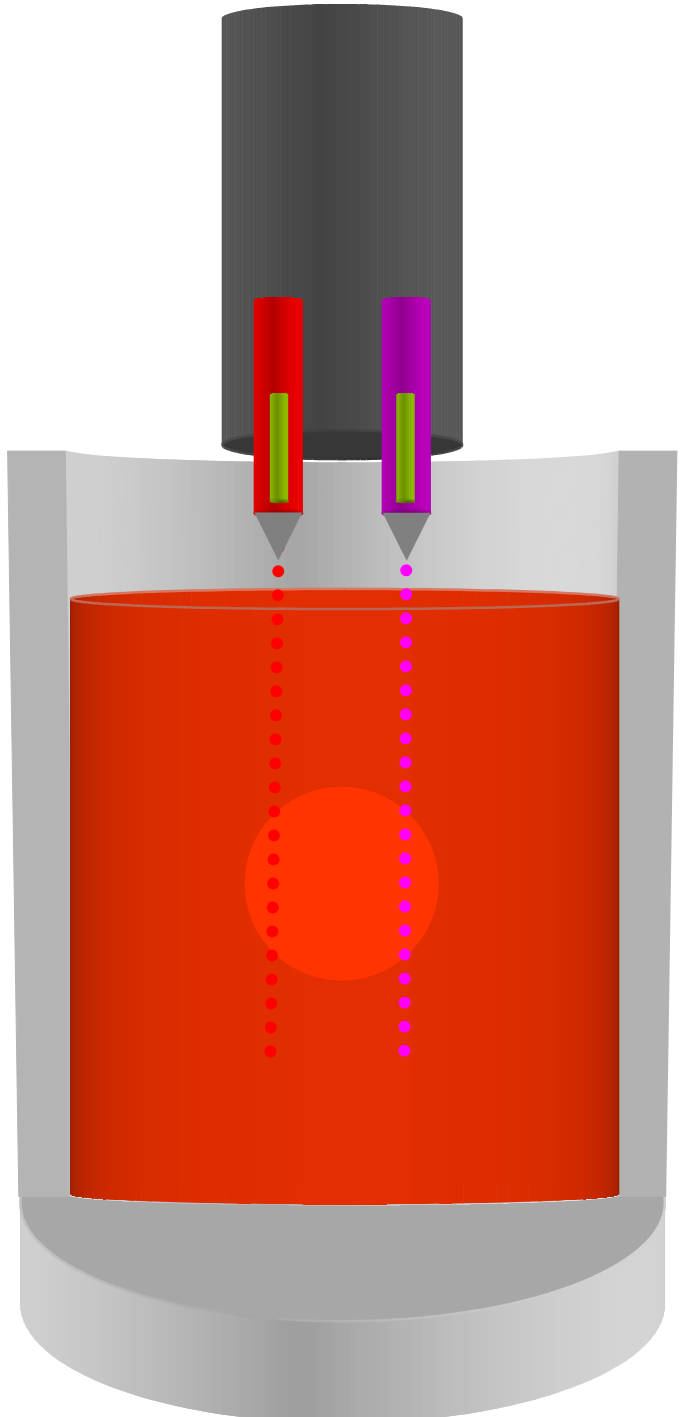
To replace the WN and SLE MAC-ELISAs with a single, equally sensitive, faster test.

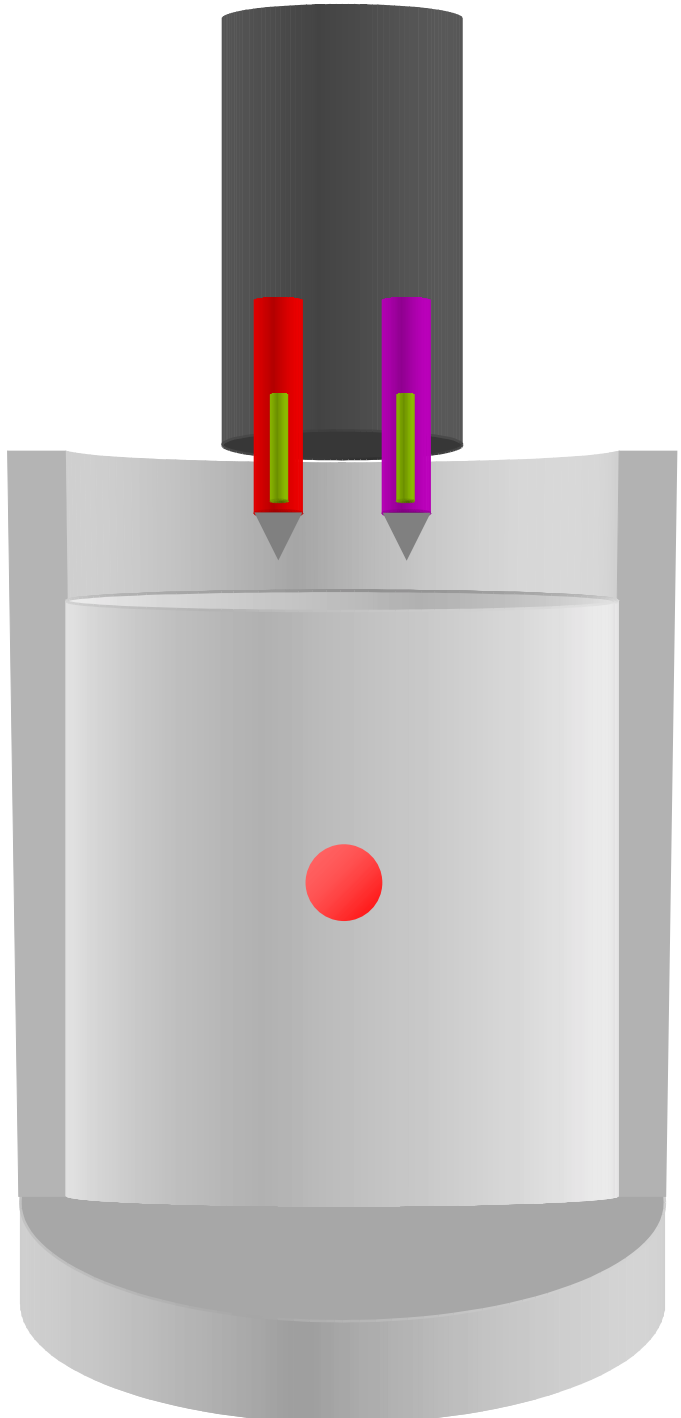
WN and SLE viruses co-circulate in parts of the US and are routinely tested for concurrently.

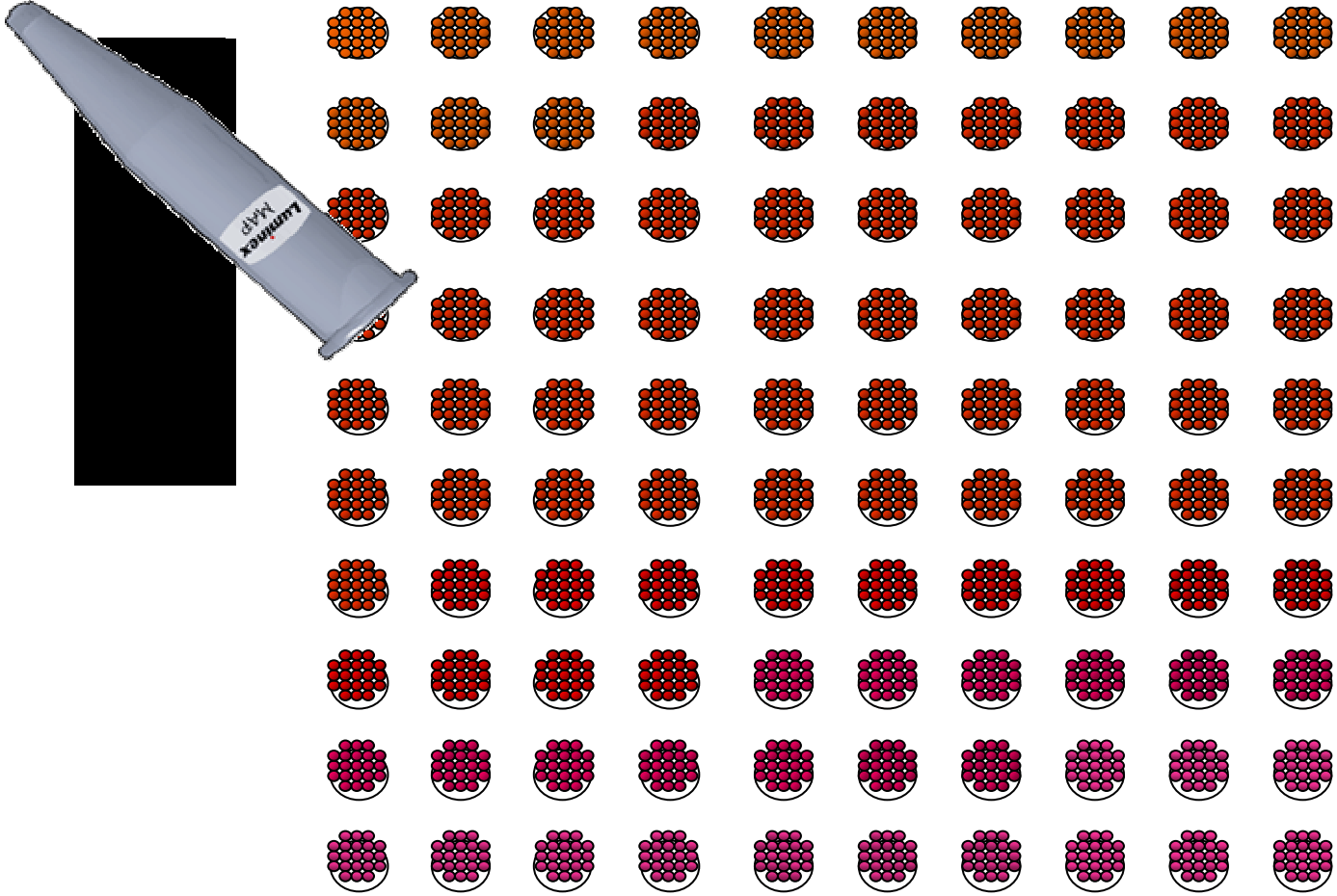
Principle

Microsphere-based immunologic assays (MIA's) are similar to ELISAs, except instead of being attached to a plate, the assay components are attached to microspheres, and results are read using a modified flow cytometer.

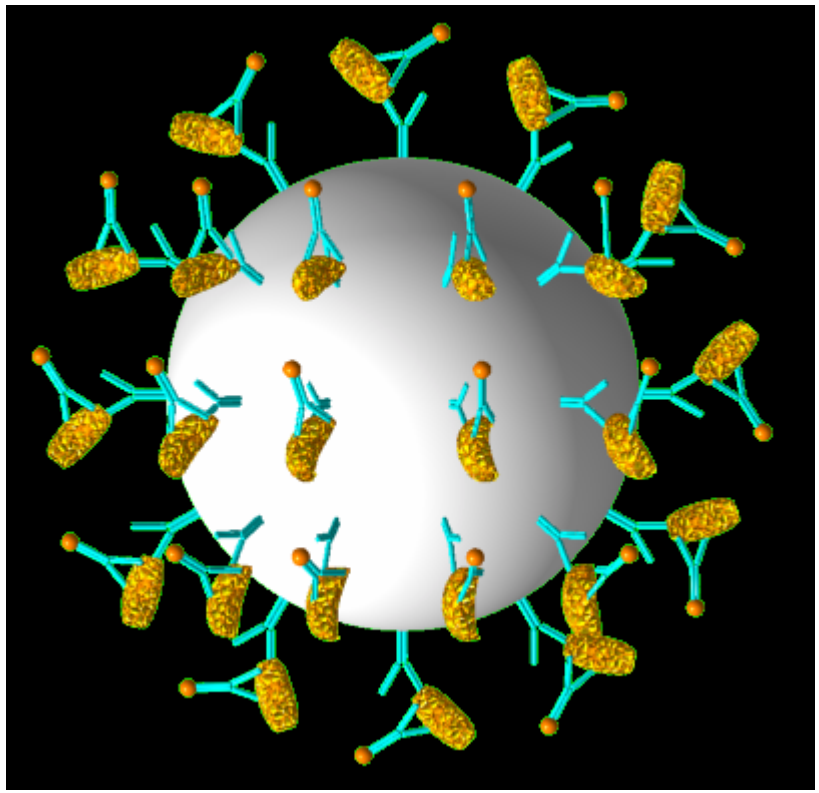
Similar problems to the ELISA are likely to arise.

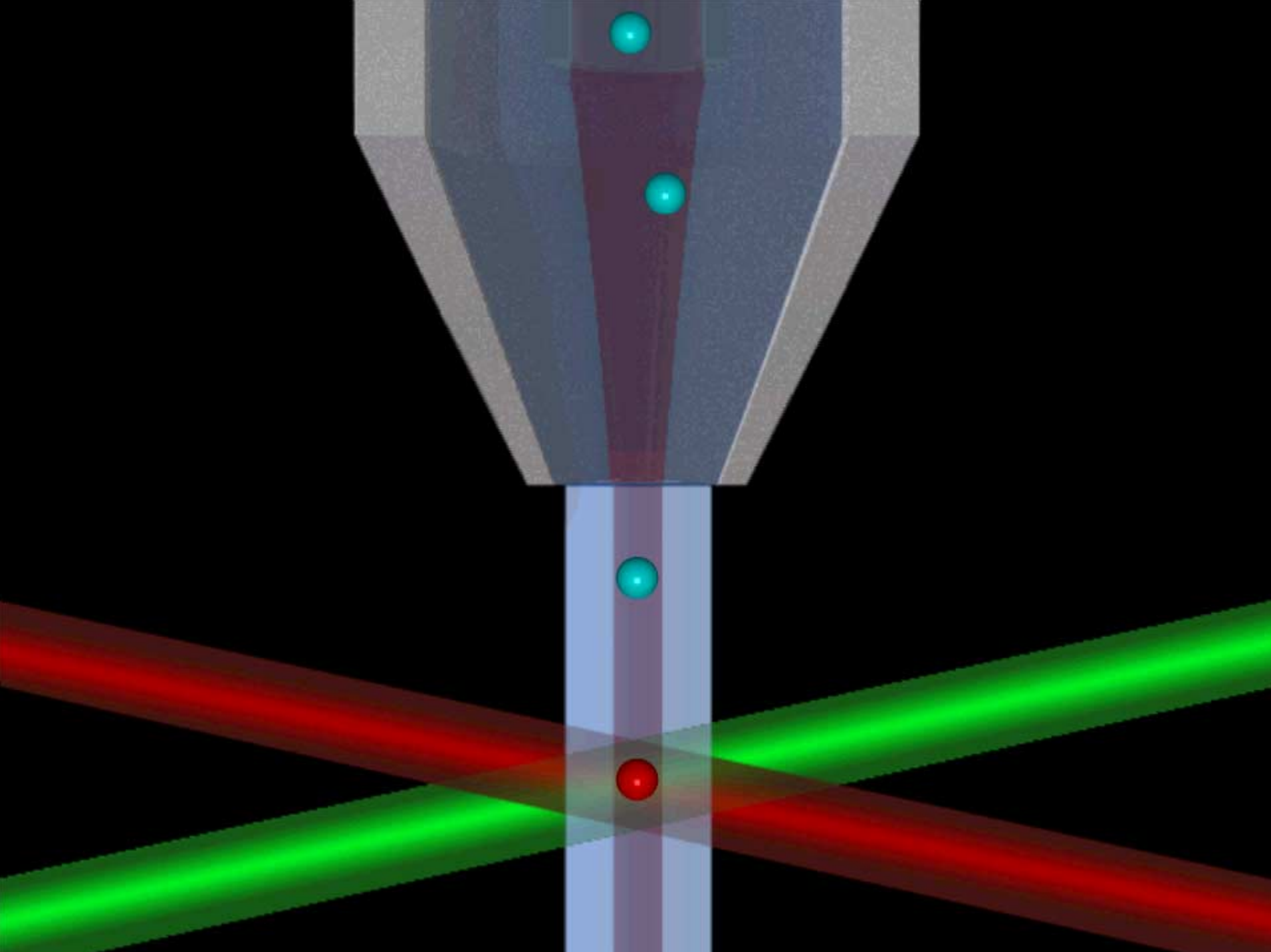


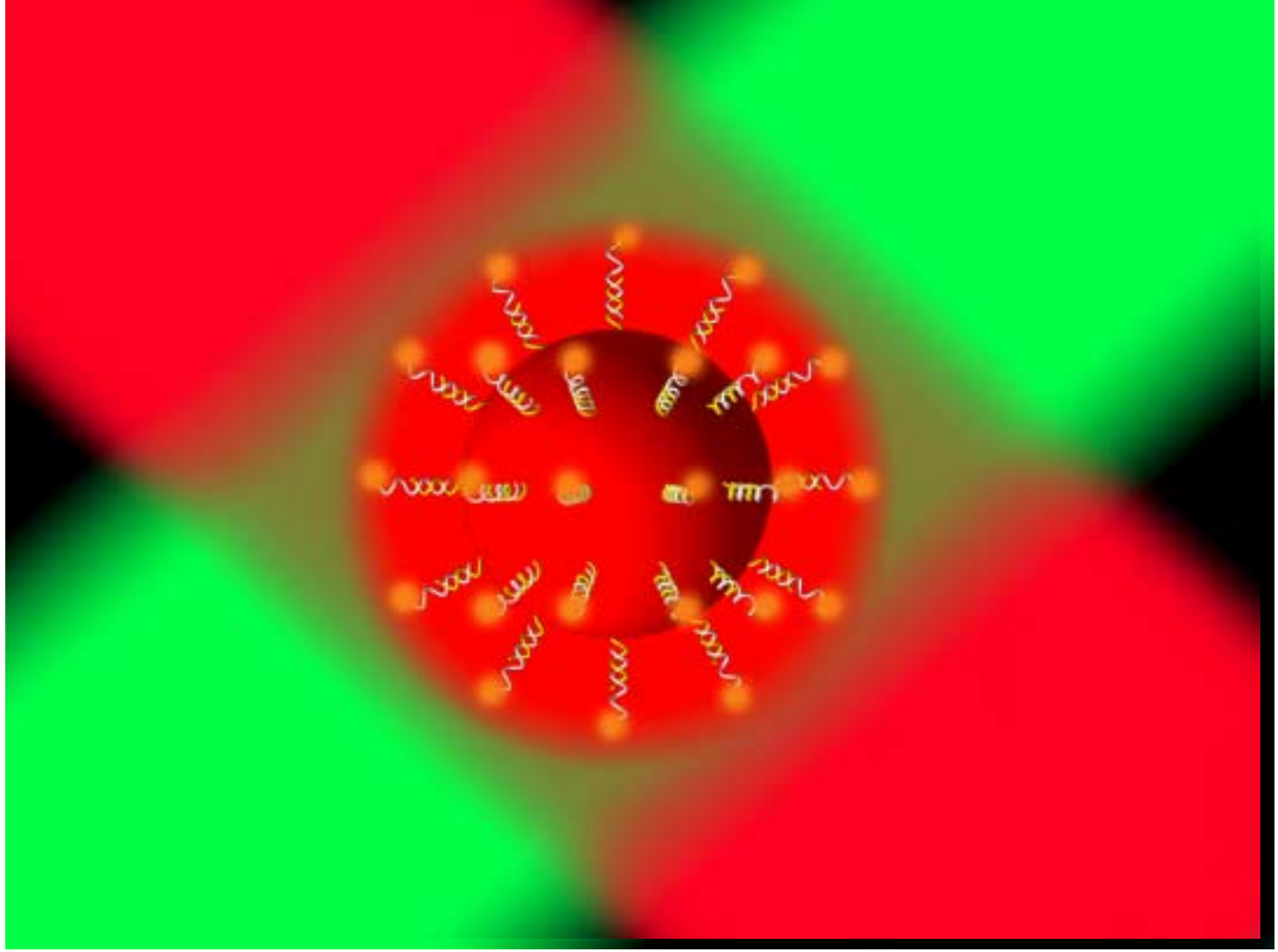




All the bead sets can be mixed in a well or a tube. The instrument sorts the data based on the unique color ratios of the beads. The individual bead sets are tagged with biological tests where the instrument identifies the set number, and measures the binding results for the test.







A green laser quantifies the surface fluorescence, which represents the biological reaction. Simultaneously, the bead sets are classified by a red laser.

WN/SLE Duplex MIA method

Prior to the test the following are prepared:

1. All serum samples and controls are IgG depleted using protein G
2. Microsphere sets 32 and 57 are coupled to 6B6C-1 (commercially, stable >1 yr)
3. Viral and negative antigens are added to each of the coupled microspheres sets (stable for 1mo)

Test procedure:

WN/SLE viral antigens/coupled beads are concurrently added to 1 set of wells; negative antigens/coupled beads are added to another set of wells on a 96-well filter plate.

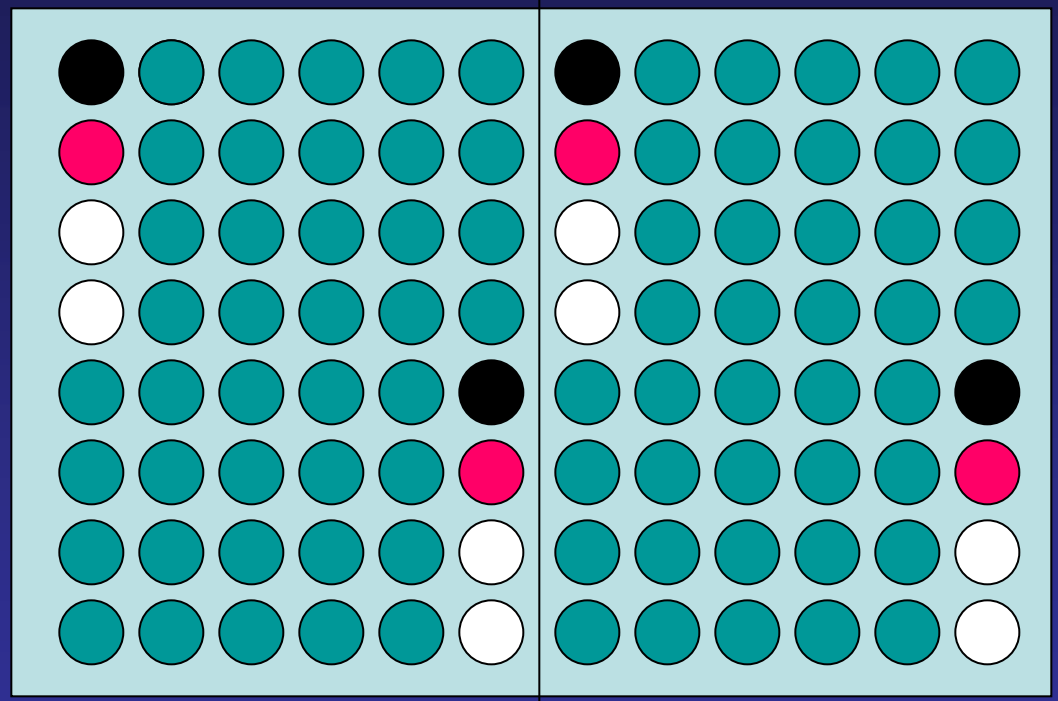
Plates are washed on vacuum manifold; serum (1:400) and anti-human IgM PE conjugate are added; mixed, and shaken for 1.5 hours.

Wells are filtered, washed, and beads resuspended. Plates are read on Luminex instrument.

Takes approx. 4.5 hours.

Typical plate format

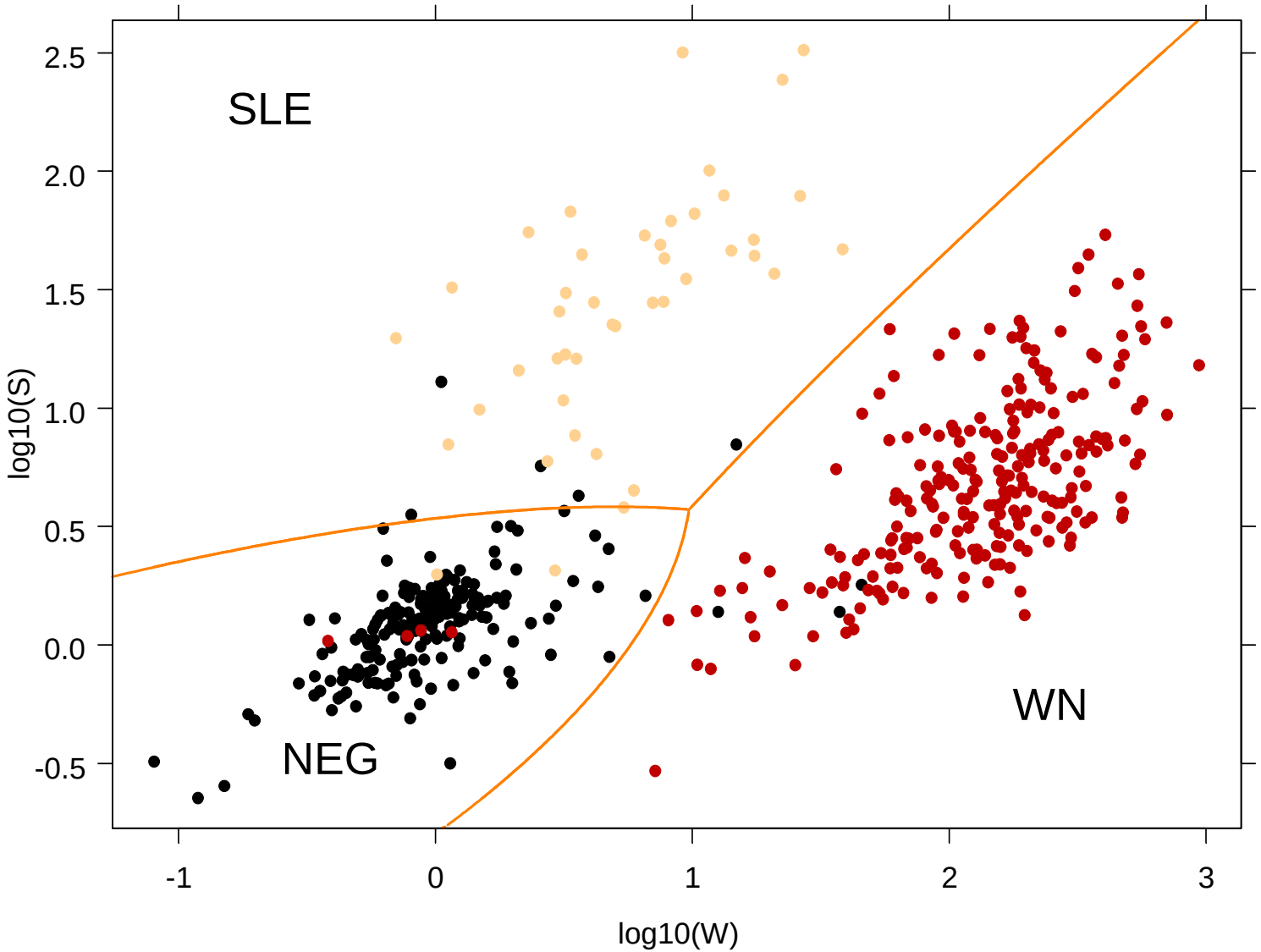
- WN pos control serum
- SLE pos control serum
- Neg control serum
- PG-treated test serum

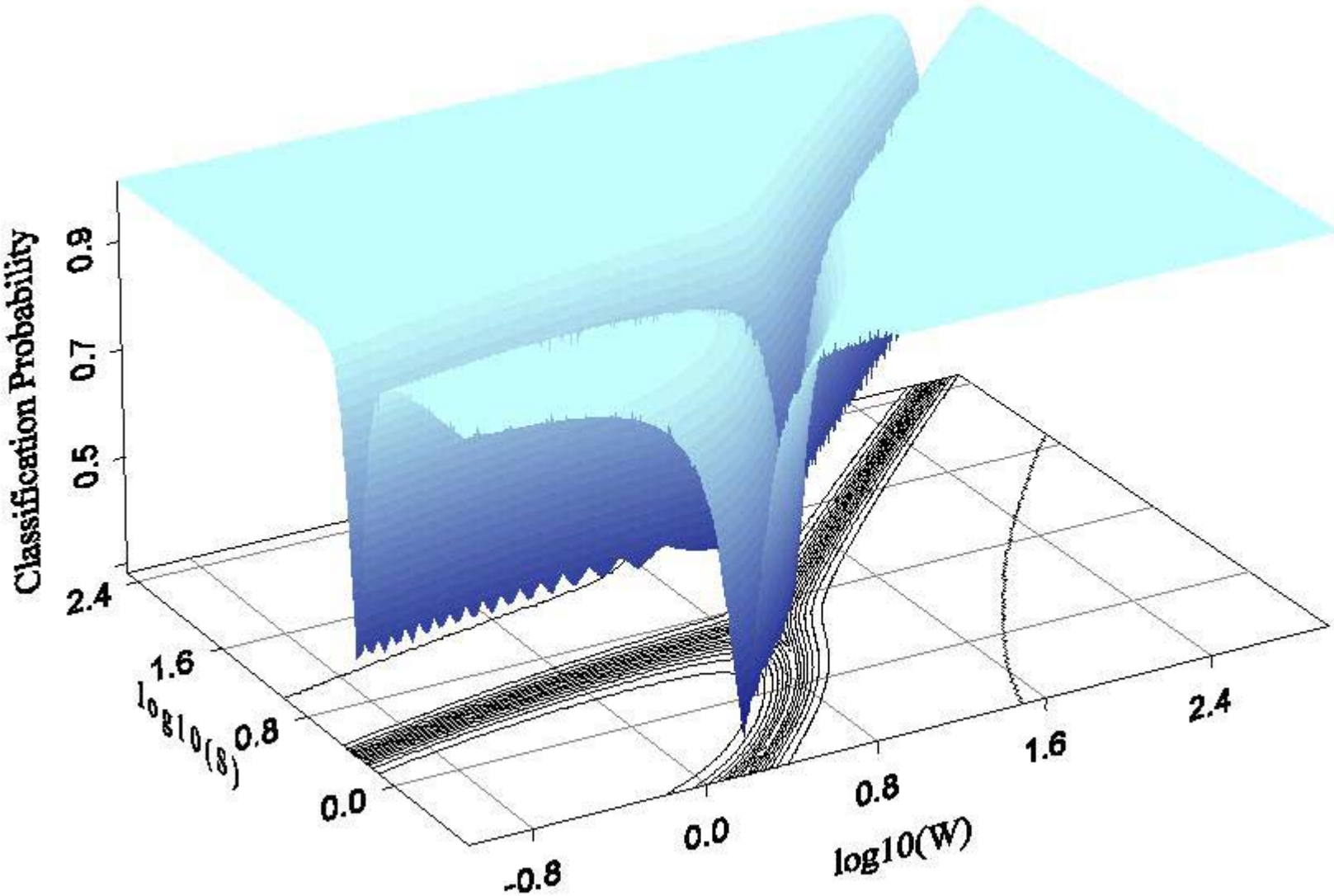


Bead set 32/WN Ag + Bead set 57/SLE Ag

Bead set 32/N Cos Ag + Bead set 57/N SMB Ag

Classification of 491 initial samples: 1. Plot log standardized WN(32) and SLE(57) values; 2. Superimpose PRNT result; 3. Apply QDA to classify. Subsequent samples are classified according to these lines.




Classification Probability
0.5
0.7
0.9
2.4
1.6
log10(8)
0.8
0.0
-0.8
0.0
0.8
1.6
2.4
log10(W)

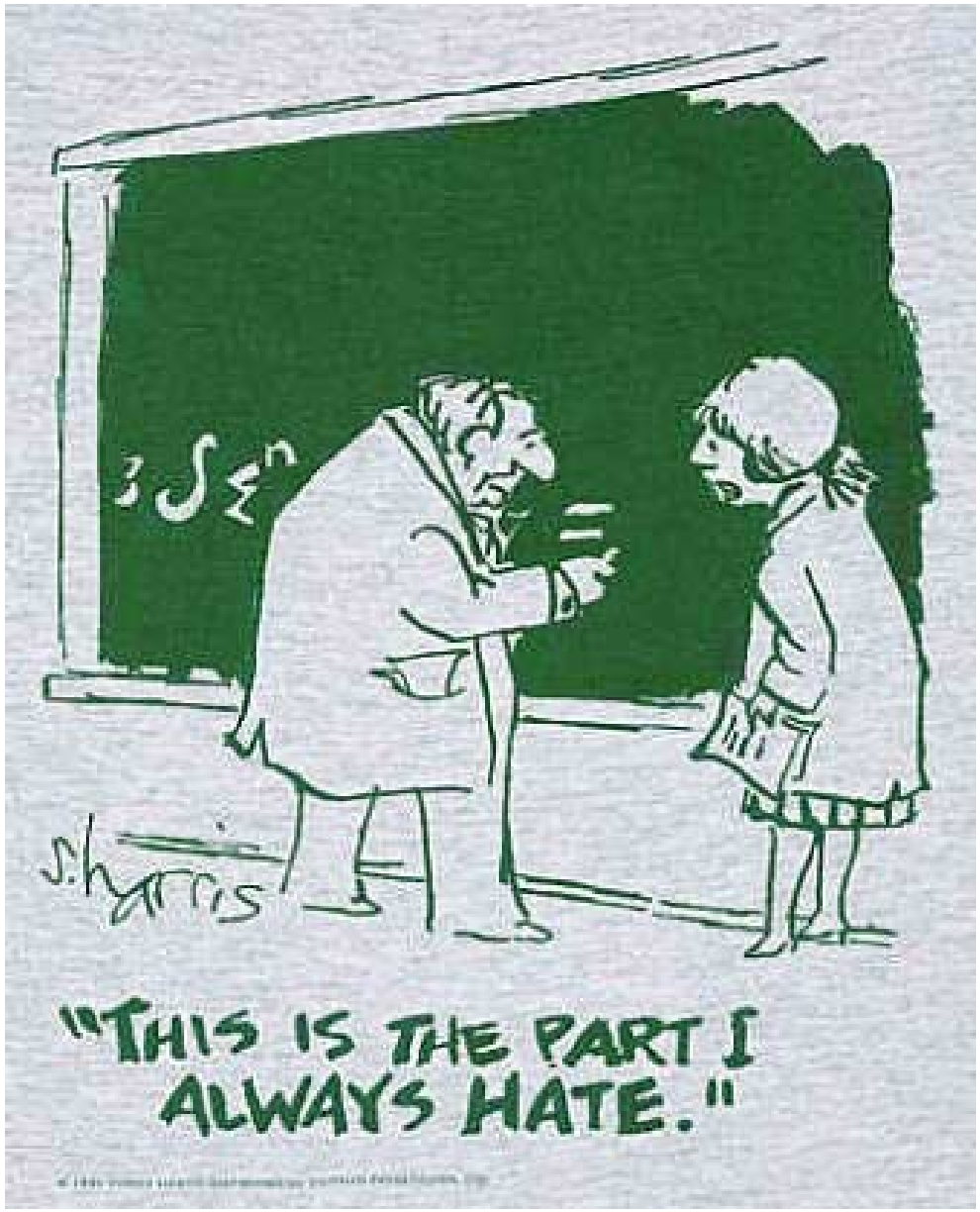
Cross-validated QDA and MAC-ELISA classifications compared to the PRNT results of 491 samples that were used to generate the QDA classification rules.

PRNT		QDA Classification			% Correct QDA Class	MAC-ELISA Classification			% Correct MAC-ELISA Class
Result	# Samples	NEG	SLE	WN		NEG	SLE	WN	
NEG	200	**192**	5	3	**96.0**	**182**	11	7	**91.0**
SLE	43	3	**40**	0	**93.0**	0	**32**	11	**74.4**
WN	248	4	0	**244**	**98.4**	5	2	**241**	**97.2**

Specificity

Antibody identity	No. of sera	MIA result			
		WN	SLE	NS	NEG
LAC	12	0	0	0	12
Old flavi	10	0	0	0	10
DEN (IgM P/N<9)	15	0	0	1	14
DEN (IgM P/N≥9)	18	1	3	5	9
YF vaccine	16	0	0	0	16
Other arbos	11	0	0	0	11
Syphilis	21	1	0	0	20
ANA	22	0	0	0	22
RF	13	0	0	0	10
LD (IgM)	10	0	0	0	10
LD (IgG)	10	0	0	0	10
NEG	154	0	0	6	148
TOTAL	312	2	3	13	294

Doing the math….


J. harris
"THIS IS THE PART I ALWAYS HATE."

Development of MIA Classify Excel Add-in software to:

- Calculate the control values
- Define constants relating to standardization of controls from all plates in development stage
- Calculate adjusted test MFIs and normalizes them according to ratio of controls
- Standardize adjusted values to historical values to allow all results to be directly compared
- Set up variables and assign values based on the QDA
- Calculate classification probabilities and produce a single result (WN, SLE or NEG)
- Identify non-specific results
- Order PRNT when necessary

ID	SLE-VAg	WN-VAg	SLE-NegAg	WN-NegAg	SLE Adj	WN Adj	SLE Adj:Con	WN Std	SLE Std
Ex WN	471	3017	19	11	24.7895	274.2727	27.0616	1.7634	0.7616
Ex SLE	1150	434	14	8	82.1429	54.2500	89.6719	1.0596	1.2819
Ex NEG	25	13	19	12	1.3158	1.0833	1.4364	-0.6401	-0.5135
Ex NS	211	92	123	83	1.7154	1.1084	1.8727	-0.6301	-0.3983
Ex WN NS	24	161	11	6	2.1818	26.8333	2.3818	0.7539	-0.2938
Ex SLE NS	208	9	21	14	9.9048	0.6429	17.8918	-0.9527	0.4918
Ex NS no Nt	373	6849	52	17	7.1731	402.8824	9.2813	1.8781	0.2414
Ex 80%Prob	17	55	15	9	1.1333	6.1111	1.2372	0.1113	-0.5783
Ex EXTRAP	14	13	326	305	0.0429	0.0426	0.0469	-2.0452	-1.9997
WN Control	54	**2194**	21	**15**	2.5476	**151.3103**	2.7811	1.5051	-0.2265
SLE Control	**2287**	137	**17**	10	**138.6061**	13.7000	151.3103	0.4619	1.5091
Neg Control	**22**	**10**	13	6	1.6863	1.5833	1.8408	-0.4752	-0.4057

ID continued	Neg Prob	WN Prob	SLE Prob	Raw Interp	Specificity	MIA Interp	Order PRNT	Extrapolation
Ex WN	0.0000	0.9999	0.0001	WN	Specific	WN	No	No
Ex SLE	0.0000	0.0001	0.9998	SLE	Specific	SLE	No	No
Ex NEG	0.9954	0.0000	0.0046	Neg	Specific	Neg	No	No
Ex NS	0.9970	0.0000	0.0030	Neg	Nonspecific	Nonspecific	Yes	No
Ex WN NS	0.0230	0.9716	0.0055	WN	WNNonspeci	Nonspecific	Yes	No
Ex SLE NS	0.0853	0.0000	0.9147	SLE	SLENonspeci	Nonspecific	Yes	No
Ex NS no Nt	0.0000	1.0000	0.0000	WN	SLENonspeci	WN	No	No
Ex 80%Prob	0.8201	0.0751	0.1048	Neg	Specific	Neg	Yes	No
Ex EXTRAP	1.0000	0.0000	0.0000	Neg	Specific	Neg	No	Yes


EASY
AS
π

How well does the test work?


YOU WANT PROOF?
I'LL GIVE YOU PROOF!
+K c'
8.1
√D
9
S. Harris

Validation

Objectives:

1. To determine the correlation between the MAC-ELISA and the MIA.
2. To determine the reproducibility of the test between labs
3. To determine if any discrepant results were associated with particular runs
4. To determine whether nonspecific parameters can be refined and whether the 80% probability difference PRNT rule needs changing

Approach:

- Trained 4 states in the method in Sept 04.
- Verified that the tests were working in each lab by use of a proficiency panel
- Each lab tested +/-200 specimens by MIA, ELISA, and PRNT when necessary
- Each sample was sent to CDC for comparative MIA testing
- 338 other samples submitted directly to CDC for WN/SLE testing were tested by MIA concurrently with other tests according to the CDC testing algorithm
- Validation only for WN and NEG samples (insufficient SLE positives available for true validation)

1. MIA vs MAC-ELISA

CDC MAC-ELISA

CDC MIA

Class *	NEG	SLE	WN	NEG	% Agree
NEG	145	3	0	148	98
SLE	0	8	0	8	100
WN	1	1	121	123	98
Total	146	12	121	279	
% Agree	99	67**	100		

* Nonspecific and equivocal results not included

** % agreement improves to 83% when compared to PRNT

MIA vs MAC-ELISA: States

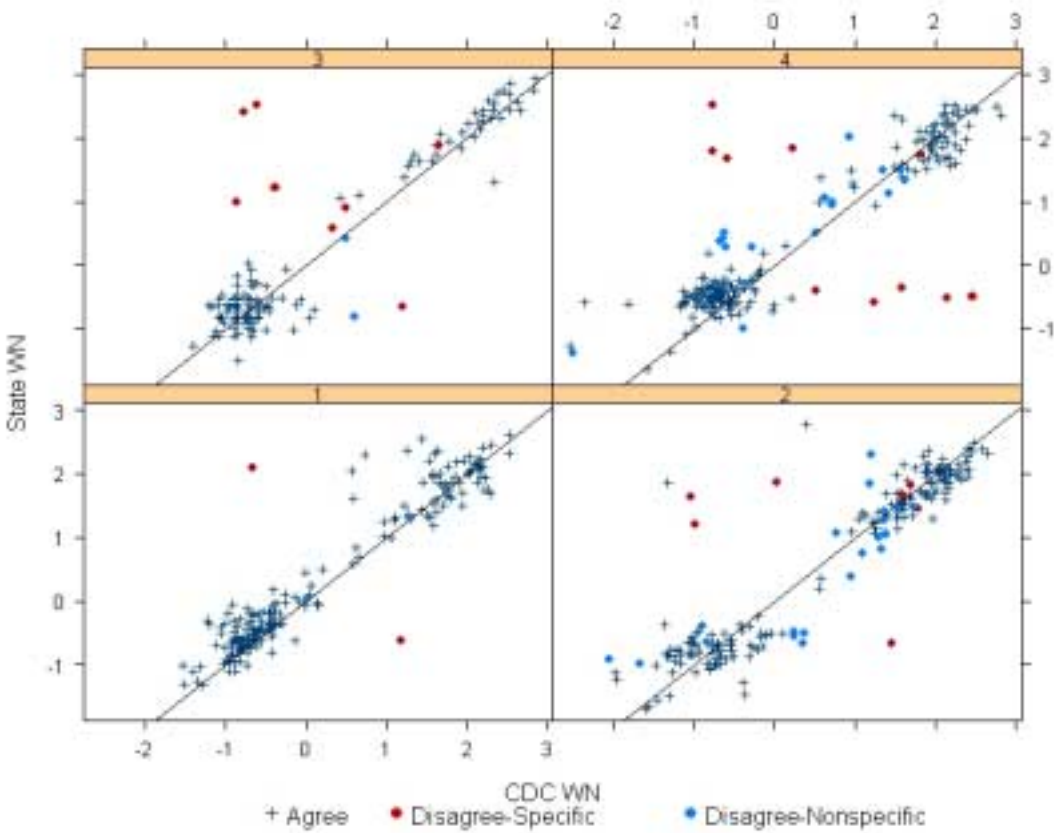
Combined States MAC-ELISA

Combined States MIA

Class*	NEG	WN	Total	% Agree
NEG	442	7	449	98
WN	25	249	274	91
Total	467	256	723	
% Agree	95	97		

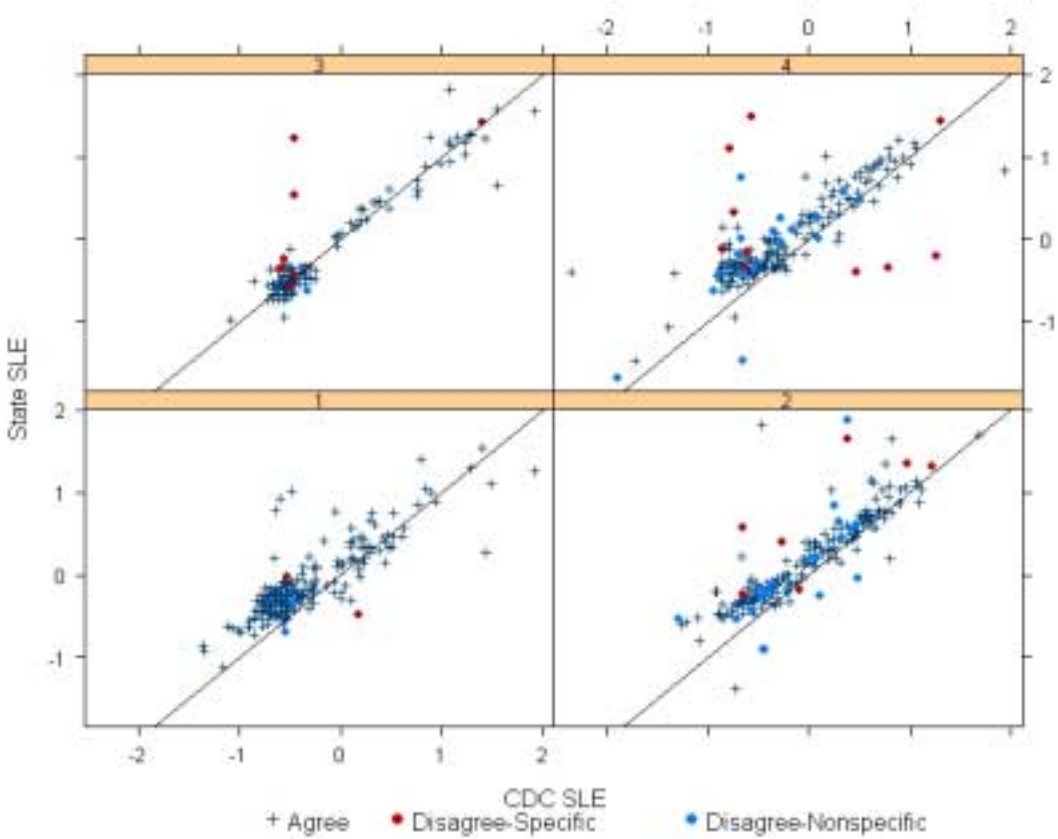
* SLE results not yet available for all states; Nonspecific and equivocal results not included.

WNV Agreement (log_{10} W comparison), State vs CDC



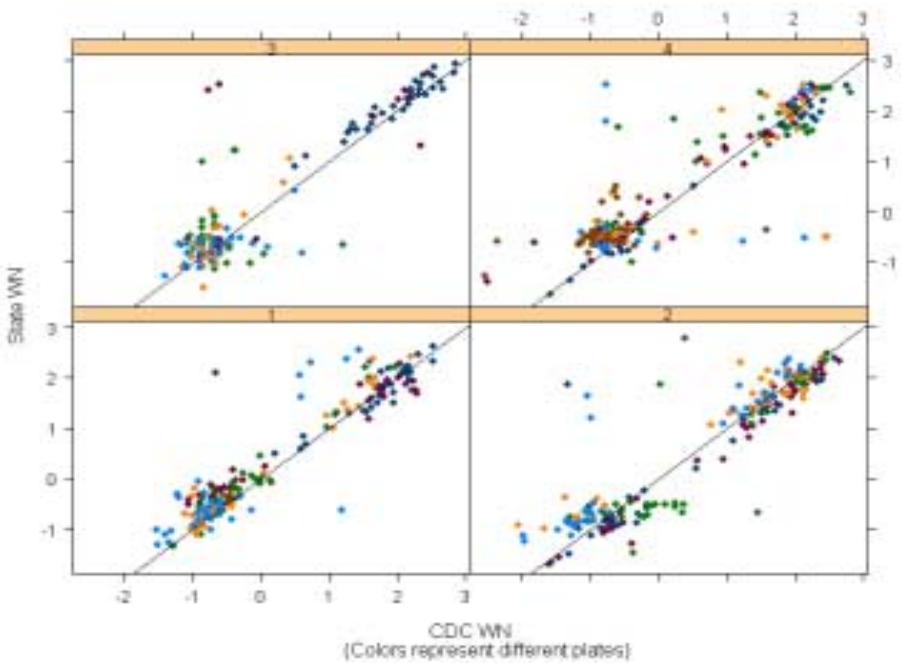
State 1
Intraclass Correlation Coefficient: 0.94;
(95% CI: 0.92, 0.95)
State 2
Intraclass Correlation Coefficient: 0.92;
(95% CI: 0.89, 0.94)
State 3
Intraclass Correlation Coefficient: 0.92;
(95% CI: 0.88, 0.94)
State 4
Intraclass Correlation Coefficient: 0.88;
(95% CI: 0.84,0.91

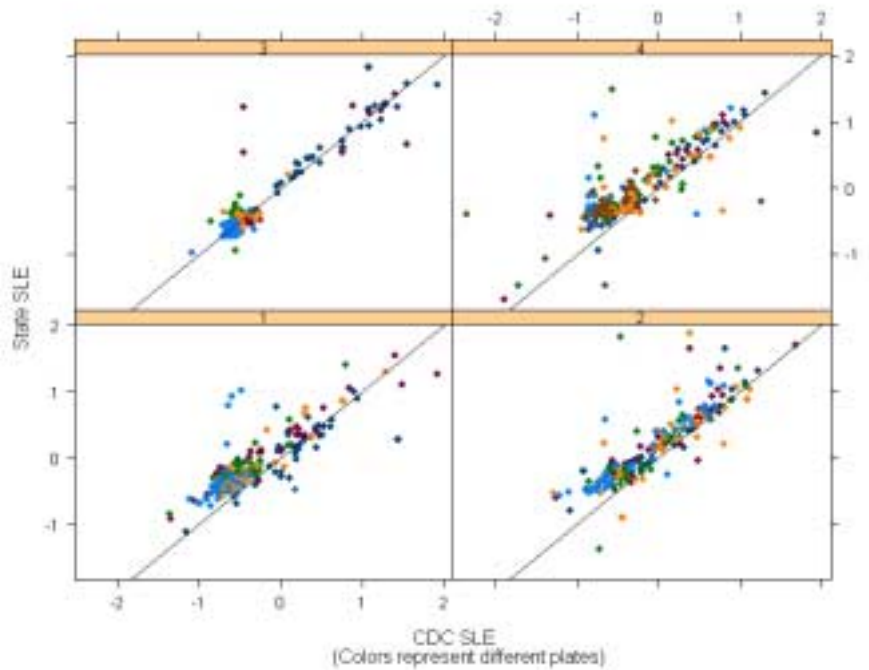
SLE Agreement (log_{10} S comparison), State vs CDC



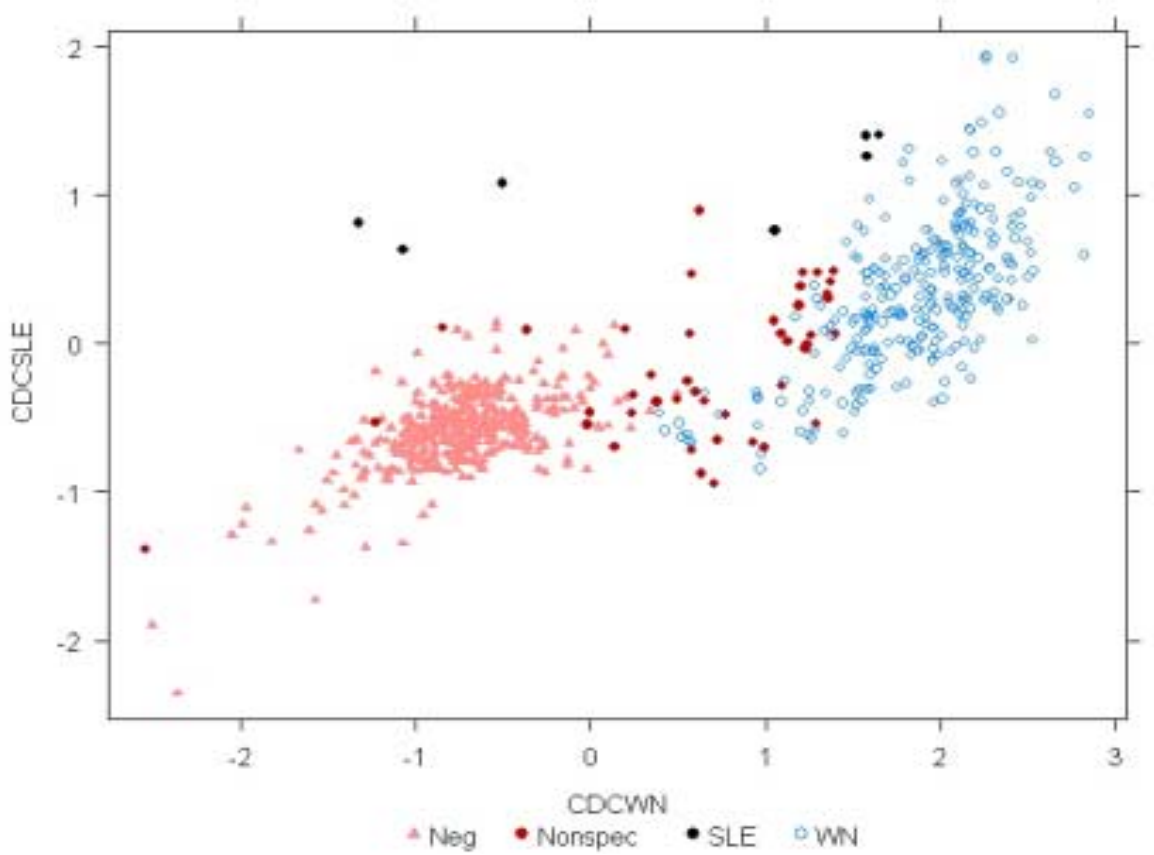
State 1
Intraclass Correlation Coefficient: 0.75;
(95% CI: 0.68, 0.80)
State 2
Intraclass Correlation Coefficient: 0.79;
(95% CI: 0.73, 0.84)
State 3
Intraclass Correlation Coefficient: 0.94;
(95% CI: 0.92, 0.95)
State 4
Intraclass Correlation Coefficient: 0.69;
(95% CI: 0.62, 0.76)

Plate distribution of results

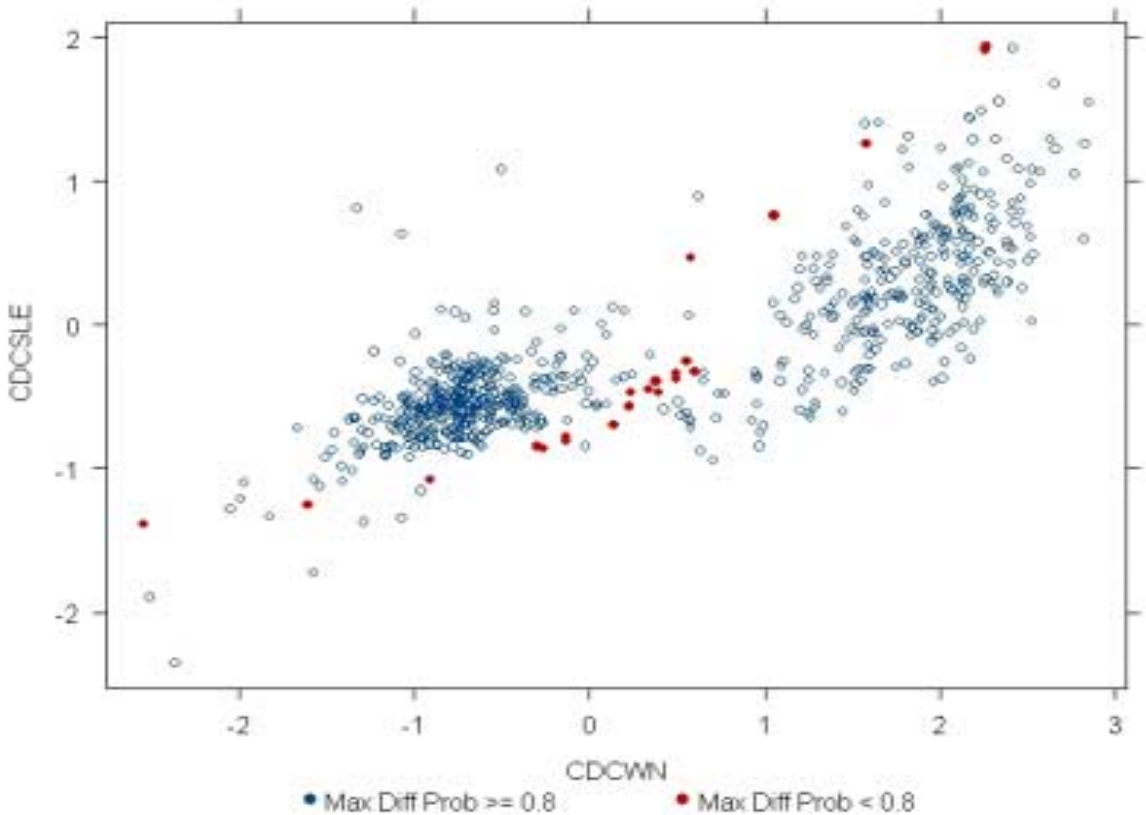




CDC MIA results for all 4 states showing nonspecific result distribution



CDC MIA results for all 4 states showing <80% probability difference result distribution



NONSPECIFICS

Nonspecifics identified by lowest MFI/probabilities for true positive samples as seen in original data set

Total # samples in validation = 1136

Total samples classified as nonspecific to one or both antigens = 102

Of the 102 nonspecific samples 78 agreed with raw MIA interpretation. Greatest disparity seen among negative raw interpretations that confirm as positives.

Indicates that nonspecific criteria can be changed, especially with regards the WN raw interpretations, to reduce the number of PRNTs performed

SLE sample numbers are too small; therefore PRNTs may be necessary for all SLE MIA-positive samples for the time being.

80% probability difference

37 (3%) of all samples were recommended for PRNT due to a maximum probability difference between groups of <80%.

15/37 were also classified as nonspecific.

Of the specific samples (22) no discrepancies between the raw MIA interpretation and the final interpretation (PRNT/ELISA) were seen above 61%.

Conclude that the 80% probability difference could be lowered

Summary

- Data transformation algorithm and software developed
- Temporary criteria to indicate PRNT confirmation determined
- In-house and external test validation projects initiated
- Test appears valid for WN and NEG samples; SLE not statistically validated due to low sample numbers

To do:

1. Obtain more SLE samples if possible; in the meantime perform PRNTs on SLE positive samples
2. Add the validation samples to the original development data and recalculate the QDA; compare resulting classifications
3. Alter data transformation software accordingly
4. Adjust nonspecific criteria in the software and possibly lower the 80% cutoff to reduce the number of PRNTs recommended

CDC	States
Brad Biggerstaff	Ron Cheshier, AZ
Preeti Gupta	Giorgio Cosentino, CA
Amanda Noga	Heather Masri, VA
Olga Kosoy	Rebecca Oesterle, MI
Janeen Laven	
Denise Martin	Focus Technologies