



# Europium Nanoparticle-Based Assays for Sensitive Detection of HIV-1 p24 Antigen

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# HIV-1 capsid protein (p24)

- Critical for virus assembly and replication.
- Shell of viral core that consists of ribonucleoprotein complex responsible for virus replication.
- Most abundant viral protein (1500-3000 p24 molecules/virion)
- High levels in the blood during early and late stages of HIV infection.
- A potential viral marker for diagnosis, blood donor screening, monitoring disease progression, and evaluating antiretroviral therapy.
- Useful for HIV diagnostics in pediatric and testing the blood supply in resource-limited settings.
- P24 and anti-p24 antibody could be new biomarkers for acute HIV infection.

# New Testing Methods for Detection of HIV-1 p24

Method	Detection Limit	Reference
Conventional ELISA	~ 10 pg/ml	
Boosted ELISA using tyramide-mediated signal amplification (TSA)	0.5 pg/ml	Schupbach J, et al. AIDS 1996;10:1085 Sutthent R, et al. J Clin Microbiol 2003; 41:1016
Gold nanoparticle-based biobarcode amplification (BCA)	0.1 pg/ml	Tang S, et al. J AIDS 2007; 46:231 Kim EY, et al. Nanomedicine 2008; 3:293
Immuno-PCR	184 ag/ml = 230 p24 / reaction	Barletta J, et al. J Virol Methods 2009;157:122
Single-molecule immunosorbent assay (SMISA)	0.1 pg/ml	Li J, et al. Anal Bioanal Chem 2009; 394:489
Immunofluorescent cytometric bead Assay	0.4 pg/ml	Biancotto A, et al. J Virol Methods 2009; 157:98
Microsphere Immunoassay with TSA	1 pg/ml	Ondoa P, et al. Cytometry Part B 2009; 76B:231
Magnetic immuno-chromatography (MICT)	15~30 pg/ml	Workman S, et al. J. Virol Methods 2009; 160:14
Carbon nanoparticle-based rapid Assay	?	Parpia Z, et al. CROI 2010; abstract

# Nanotechnology & Nanomaterials

- Research at atomic, molecular, or macromolecular scale, leading to controlled creation and use of structures, devices, and systems with a length scale of 1-100 nanometers (nm).
- At the nanoscale, physical, chemical, and biological properties of materials differ fundamentally from those of the corresponding bulk material e.g.

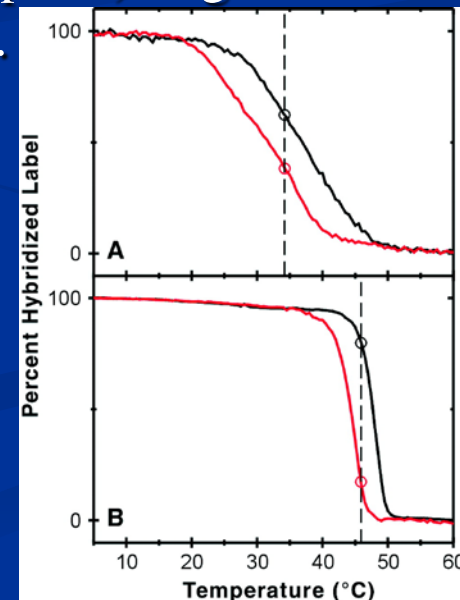
# small size and large surface-to-volume ratio;

# chemically tailorable physical properties;

# unique physical properties - very sharp melting point, magnetic properties, and size-dependent absorption (color).



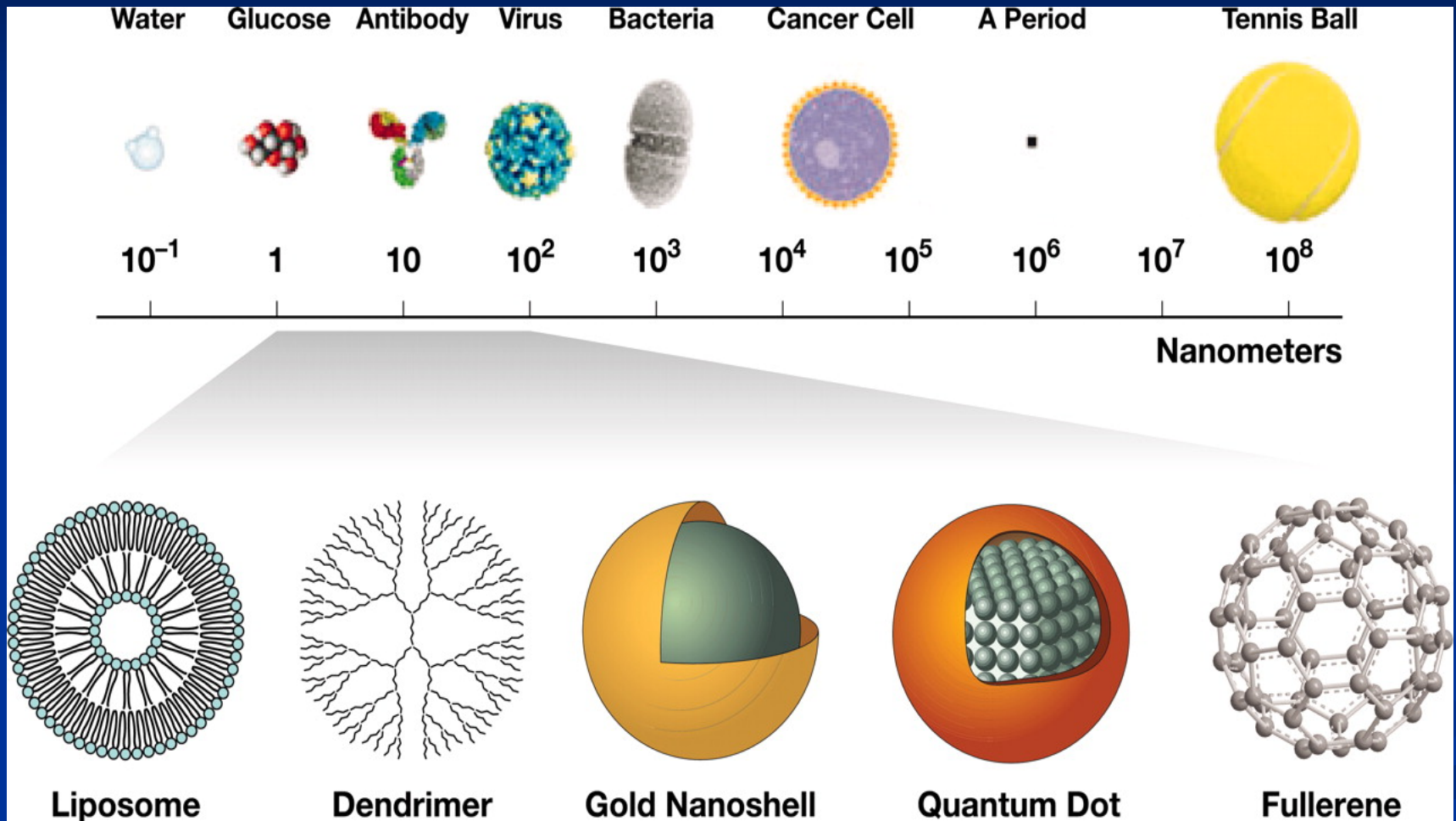
20 70 100 146 182 240 nm  
size-distinguishable selenium NPs



# Nano-scale Diagnostics

- Nanotechnology-based approaches could potentially provide a new generation of diagnostic assays.
- Nanotechnology offers some potentially unique features that could permit rapid, sensitive detection of multiple pathogens and analytes simultaneously
- Nano-scale detection could permit miniaturization of testing allowing testing of small volumes of sample with a high degree of sensitivity

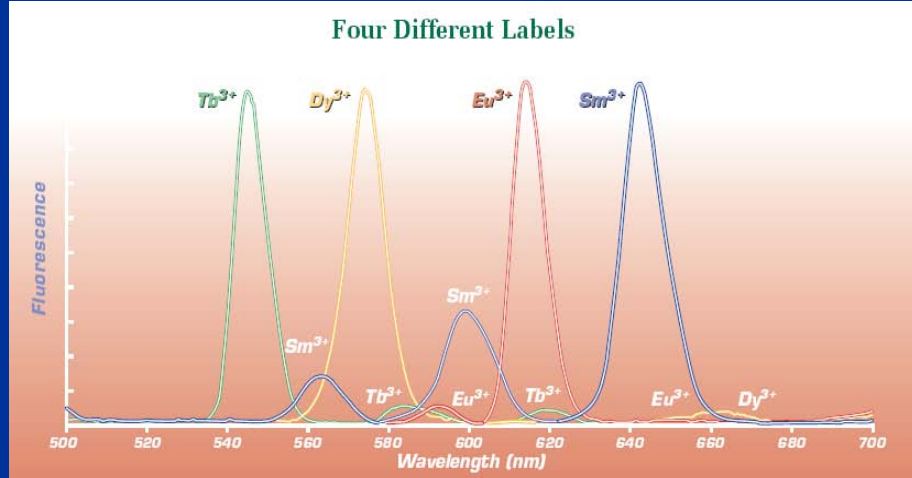
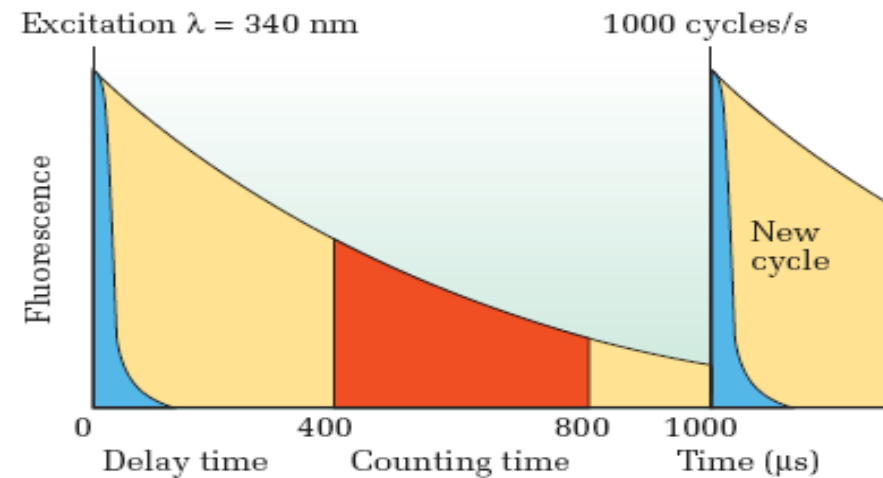
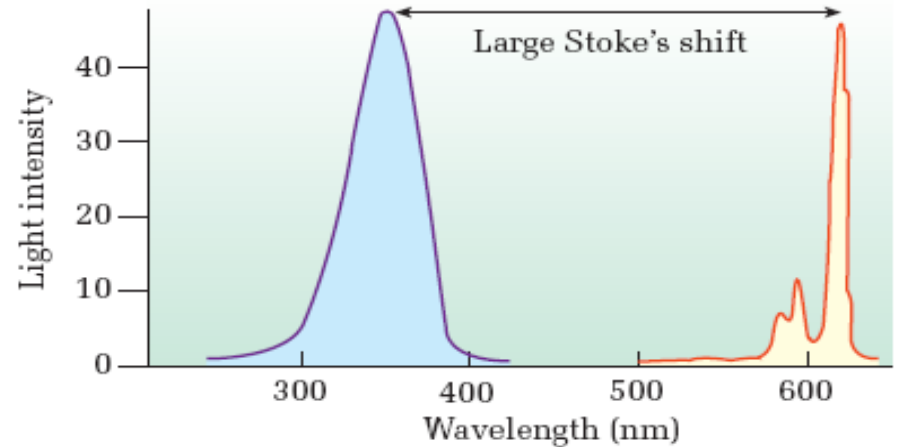
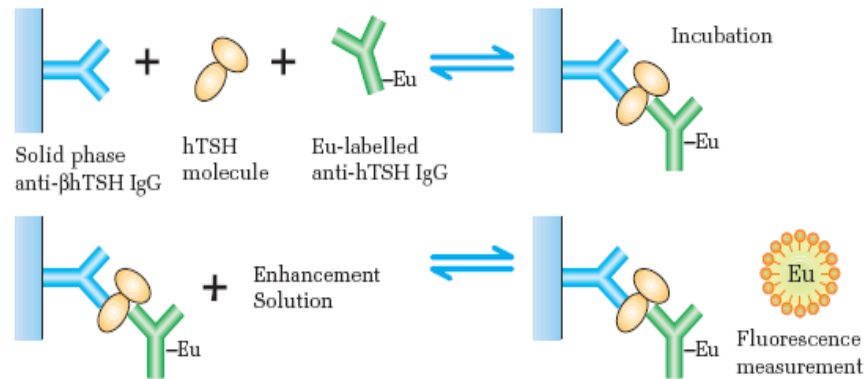
# Nanoparticles





# Europium Chelates

*DELFLIA hTSH, an example of a DELFLIA non-competitive sandwich type assay.*

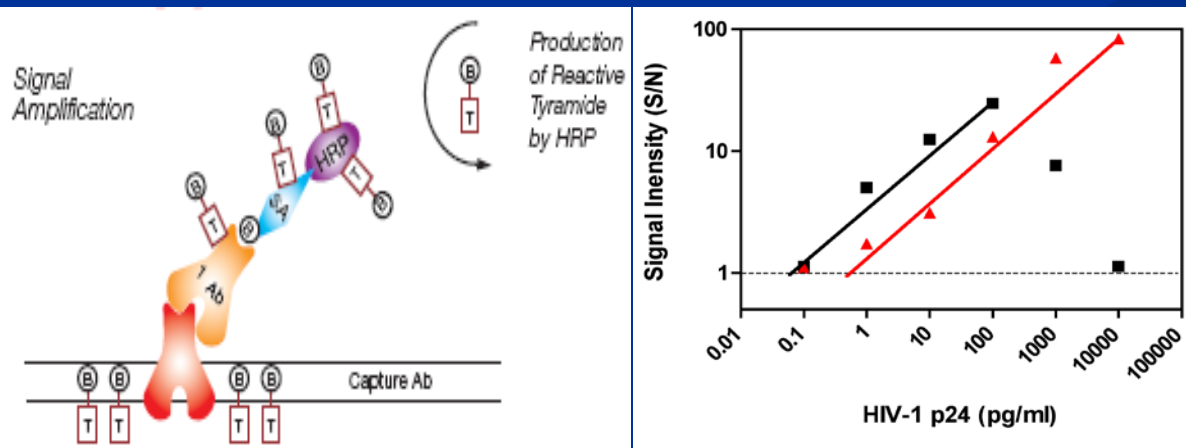
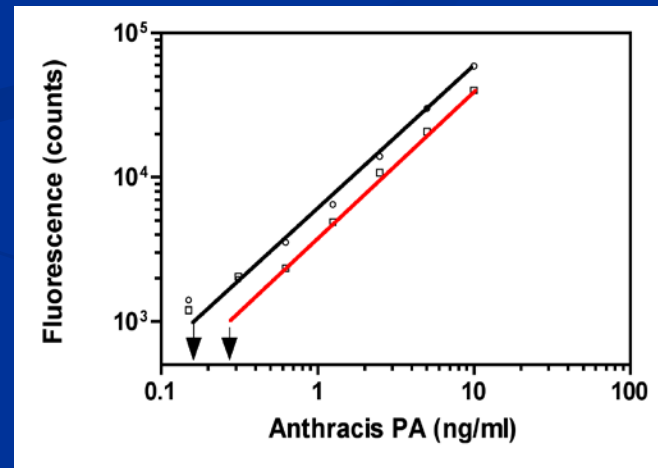
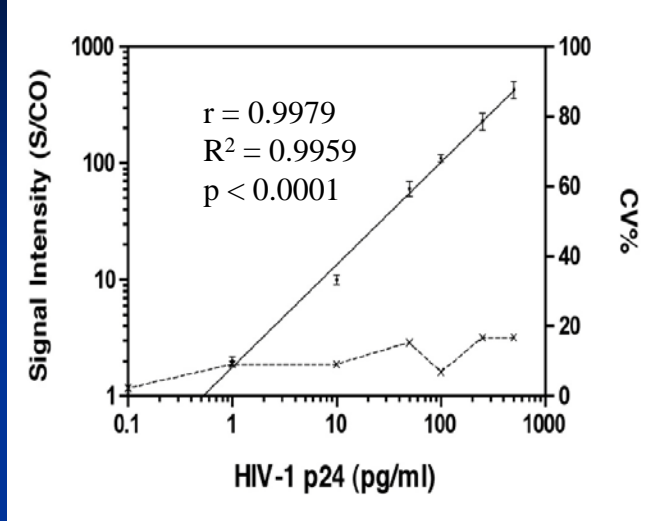
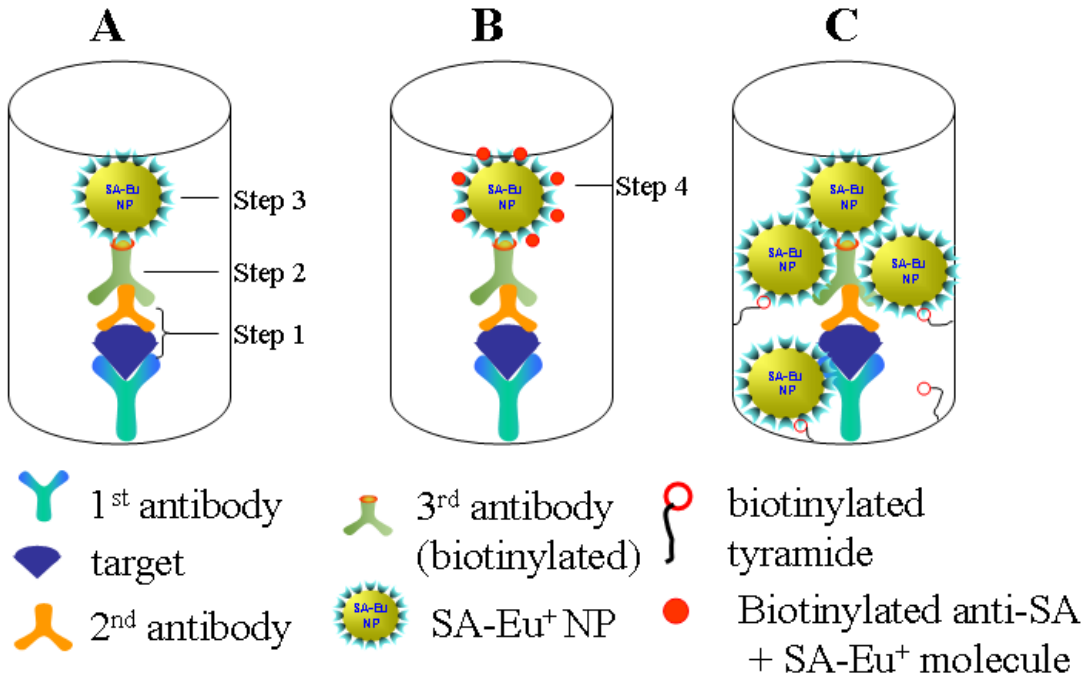


# Europium Nanoparticle

- SA labelled polystyrene  $\text{Eu}^{3+}$  nanoparticles:
  - 107 nm, containing  $\text{Eu}^{3+}$   $\beta$ -diketone chelates
  - 31000 europium ions per nanoparticle
  - 700 SA molecules per nanoparticle
- $\text{Eu}^{3+}$  NPs are much more stable against photobleaching than organic dyes.
- Low background, especially in time-resolved fluorescence (TRF) mode decreases background autofluorescence



# Scheme of $\text{Eu}^{+}$ NP-based immunoassay (ENIA)



Tang S and Hewlett IK. Chemistry Today 2009; 27(6): 50

# ENIA Is More Sensitive Than ELISA

Assay	ENIA 30ul	Original p24 assay <sup>a</sup>	In-house ELISA 30ul	PerkinElmer	
				30ul	100ul
P24 pos. (%)	32/37 (86.5)	23/37 (62.2)	19/37 (51.4)	18/37 (48.6)	20/37 (54)

<sup>a</sup> Innogenetics assay

# ENIA Can Detect HIV-1 p24 Earlier Than ELISA

PCR	Number	ENIA		In-house ELISA	
		Positive	negative	Positive	Negative
Positive	11	9 (82%)	2 <sup>b</sup>	7 (64%)	4 <sup>c</sup>
Negative	8	0	8	0	8
Total	19	9	11	7	12

- Totally 38 samples representing the duplicates of the 19 samples were tested. The results were identical for the duplicates.
- One sample (1006-06 or 13A/2B) was the first PCR positive bleed, viral load < 100 copies/ml. The other sample (1001-06 or 3A/13B) was the bleed of 7 days after PCR positive, viral load was 2500 copies/ml.
- Except the 2 false negative in “b”, the other 2 samples are: 1056-09 or 9A/17B, day 7, 65,000 copies/ml; 1057-10 or 14A/5B, day 32, 50,000 copies/ml.

# Detection of HIV-1 p24 in Dry Blood Spot (DBS) Samples by ENIA

Sample	S/N Ratio		HIV-1 p24 (pg/ml)
	w/o BT	with BT	
DBS	15	60	625
DBS	2.9	5.3	63
p24 control	64	77	100
	7.6	31	10
	1.7	2.2	1

# ENIA for Sensitive Detection of *B. anthracis* Toxin

Target	Capture Ab (ug/ml)	2 <sup>nd</sup> Ab 1:10,000	LOD (pg/ml)	
			Eu Assay	ELISA
PA	14B7 (1.0)	Rabbit anti-PA	10	1000
	W1 (1.0)	Rabbit anti-PA	10	1000
LF	LF#10 (1.0)	Goat anti-LF	10	1000
EF	PA63 (6.0)	Goat anti-EF	10000	

Dosage (spores/mouse)	Bleed Time (hours)	Animal Number	Illness grade	PA in Blood (ng/ml)
10 <sup>3</sup>	24 ~ 237	13	(-)	0.00
10 <sup>7</sup>	6	2	(-)	0.00
10 <sup>7</sup>	8	2	(-)	0.00
10 <sup>7</sup>	24	2	(+) Edema	68 ± 75
10 <sup>7</sup>	42-48	2	(+++)	408 ± 275
0	24	2	(-)	0.00

# ENIA for Sensitive Detection of *Y. pestis* F1 and LcrV Antigens

Target	Capture Ab (ug/ml)	2 <sup>nd</sup> Ab 1:10,000	LOD (pg/ml)	
			Eu Assay	ELISA
F1	YFP19 (1.25)	Rabbit antiF1	3	150
	#6031 (2.5)	Rabbit antiF1	3	75
LcrV	Va13 (5)	Rabbit antiV	30	300
	Va48 (1.25)	Rabbit antiV	1	50

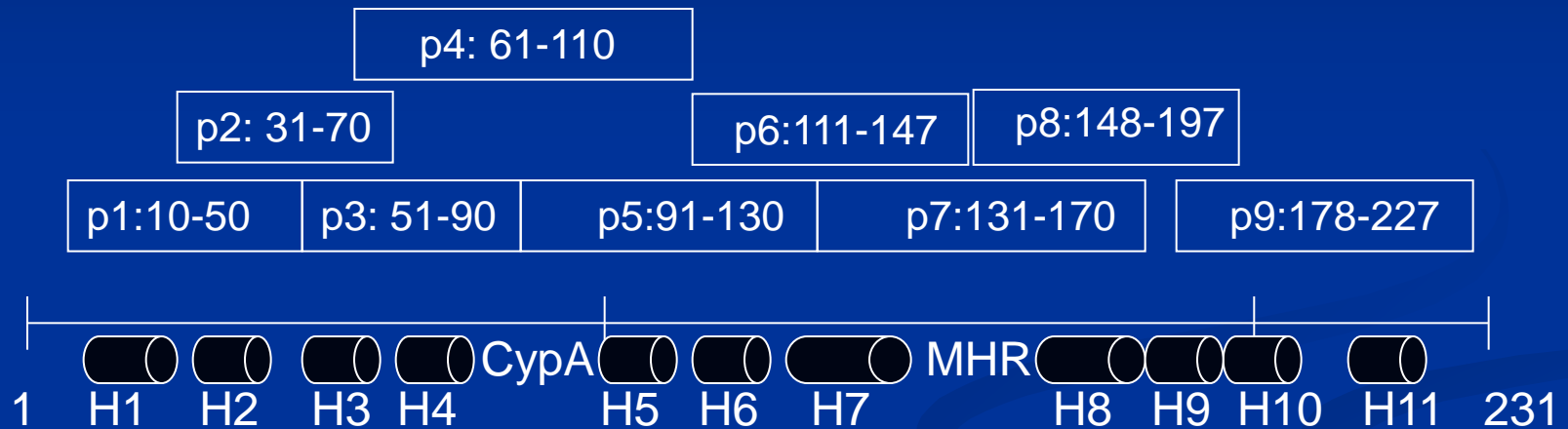


# Summary

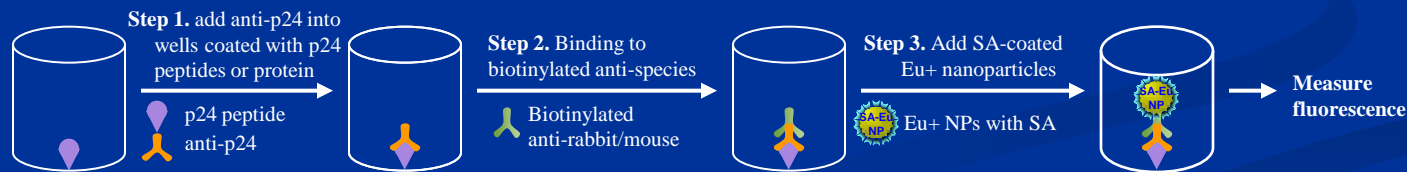
- ENIA assay could detect 0.1~0.5 pg/ml of HIV-1 p24 antigen compared with 10-15 pg/ml by conventional ELISA, and was significantly more sensitive than ELISA.
- Using this assay, good linear correlation was observed between the amounts of p24 and signal intensity, making a semi-quantitative assay.
- More than 80% of HIV-1 RNA positive samples were p24 positive by ENIA while about 50~60% was p24 positive by ELISA.
- ENIA could detect HIV-1 p24 earlier than ELISA.

# Immune Responses to HIV-1 p24 Antigen --- Implications for Detection (Poster #43)

## A. HIV-1 p24 peptides



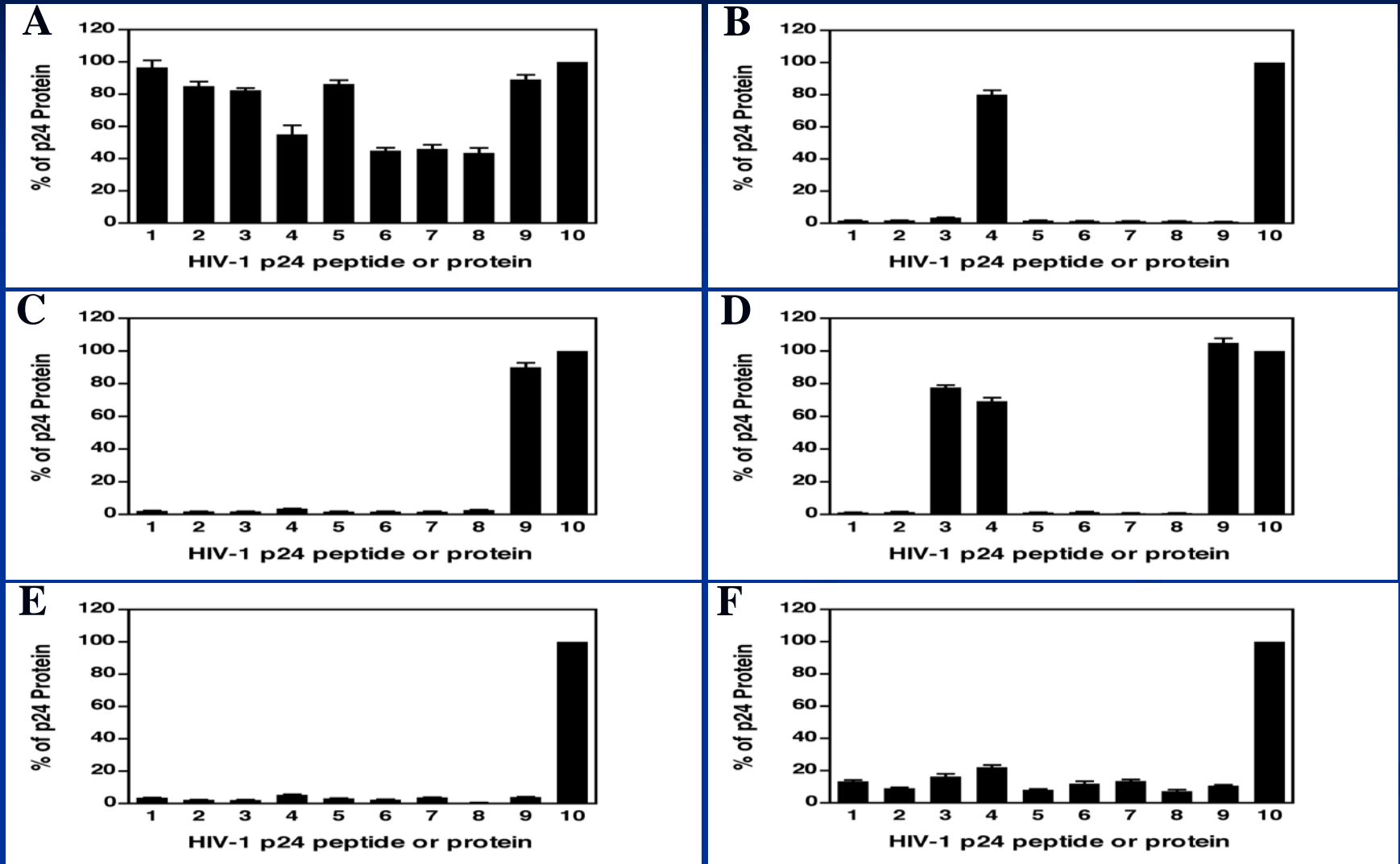
## B. p24 peptide-based immunoassay



# Characterization of MAbs anti-HIV-1 p24 antibodies

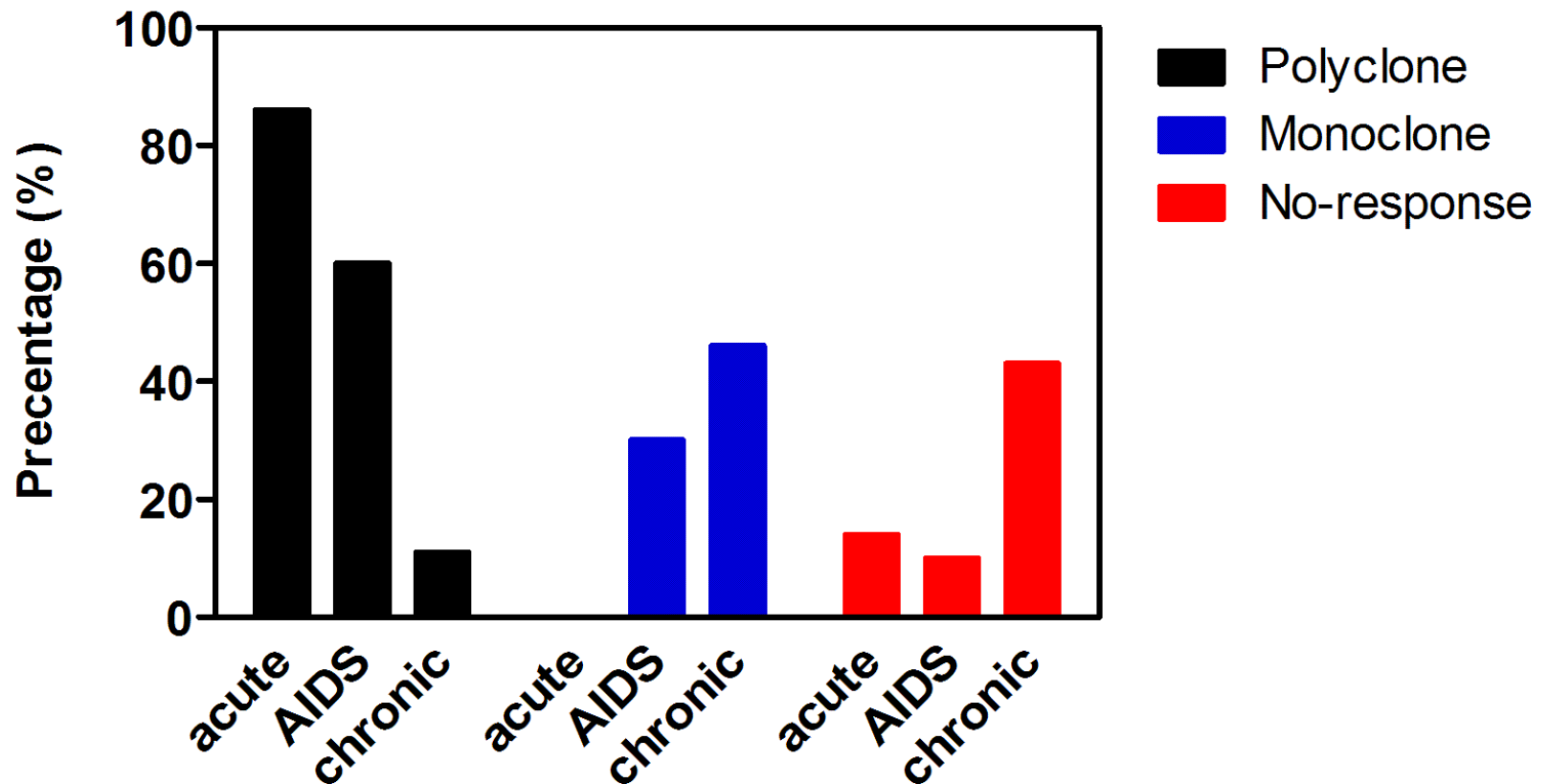
ID	Immunogen	Clone	Isotype	Reacting peptide	
#6521	P24 (HXB-3)	#24-2	IgG <sub>2b</sub> κ	p9: aa178-227	P8/9
13B6	unknown	13B6	IgG <sub>1</sub> κ	p9: aa178-227	
C65941M	unknown	#491	IgG <sub>1</sub> λ	p9: aa178-227	
20-272-19776	Recombinant p24	#473	IgG <sub>1</sub>	p9: aa178-227	
#3537	unknown	183-H12-5C	IgG <sub>1</sub> κ	p8: aa148-197	
ANT-152	Recombinant p24	YDHIV gp24	IgG <sub>1</sub>	p8: aa148-197	
SC-73300	Recombinant p24	YDHIV gp24	IgG <sub>1</sub>	p8: aa148-197	P2/3/4
C86243M	C-terminal peptide	ND1	IgG <sub>1</sub>	P3/4: aa51-110	
NB500-473	C-terminal peptide	ND1	IgG <sub>1</sub>	P3/4: aa51-110	
C65489M	p24 protein	BDI489	IgG <sub>1</sub>	p2: aa31-70	
AS55-10	Recombinant p24	MX-0316	IgG <sub>1</sub>	p2: aa31-70	none
#1103	HIV-1 IIIB p24	unknown	IgG	none	
#012-A	Recombinant p24	1A1	IgG <sub>1</sub>	none	
20-511-241432	unknown	BDI690	IgG <sub>1</sub>	none	
C65690M	unknown	BDI690	IgG <sub>1</sub> κ	none	
13G4	unknown	13G4	IgG <sub>1</sub> κ	none	

# Immune response to HIV-1 p24 during natural HIV-1 infection



**A: Polyclone-like; B, C and D: Monoclonal-like; E, F: No response or response to conformational epitopes**

# Immune Response to HIV-1 p24 during HIV-1 Natural Infection



# Summary

- The peptide-based immunoassay was simple, rapid and specific for determining immune dominant epitopes of HIV-1 p24 antigen, and for investigating immune response to HIV-1 p24 during natural HIV-1 infection.
- Two major epitope regions which locate at CypA binding loop and adjacent helices and end of C-terminal domain were found by characterization of monoclonal anti-p24 antibodies and by analysis of HIV-1 positive sera.
- Different immune response patterns were observed in HIV-1 positive sera, and indicate a clear switch of immune response to the peptides of HIV-1 p24 from polyclone-like pattern during acute HIV infection to monoclonal-like or no response patterns during chronic infection of HIV.
- The further identification of epitopes of HIV-1 p24 that are specific for distinguishing acute and chronic HIV-1 infection may help to develop new biomarkers or methods for the diagnosis of recent HIV-1 infection.
- Although HIV is highly divergent virus, anti-HIV p24 antibodies show broad cross-reactivity with different viruses upon the quality of the antibodies. The combination of anti-p24 antibodies targeting to different epitopes can significantly improve the detection sensitivity.
- These results provide the foundation for development and refinement of testing assays for detection of HIV-1 p24 antigen.



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