Rapid detection of HIV-1 p24 antigen and of antibodies to HIV-1 and HIV-2 using magnetic immuno-chromatography (MICT)

Timothy C. Granade, MS

HIV Laboratory Branch

Division of HIV/AIDS Prevention

NCHHSTP/CDC

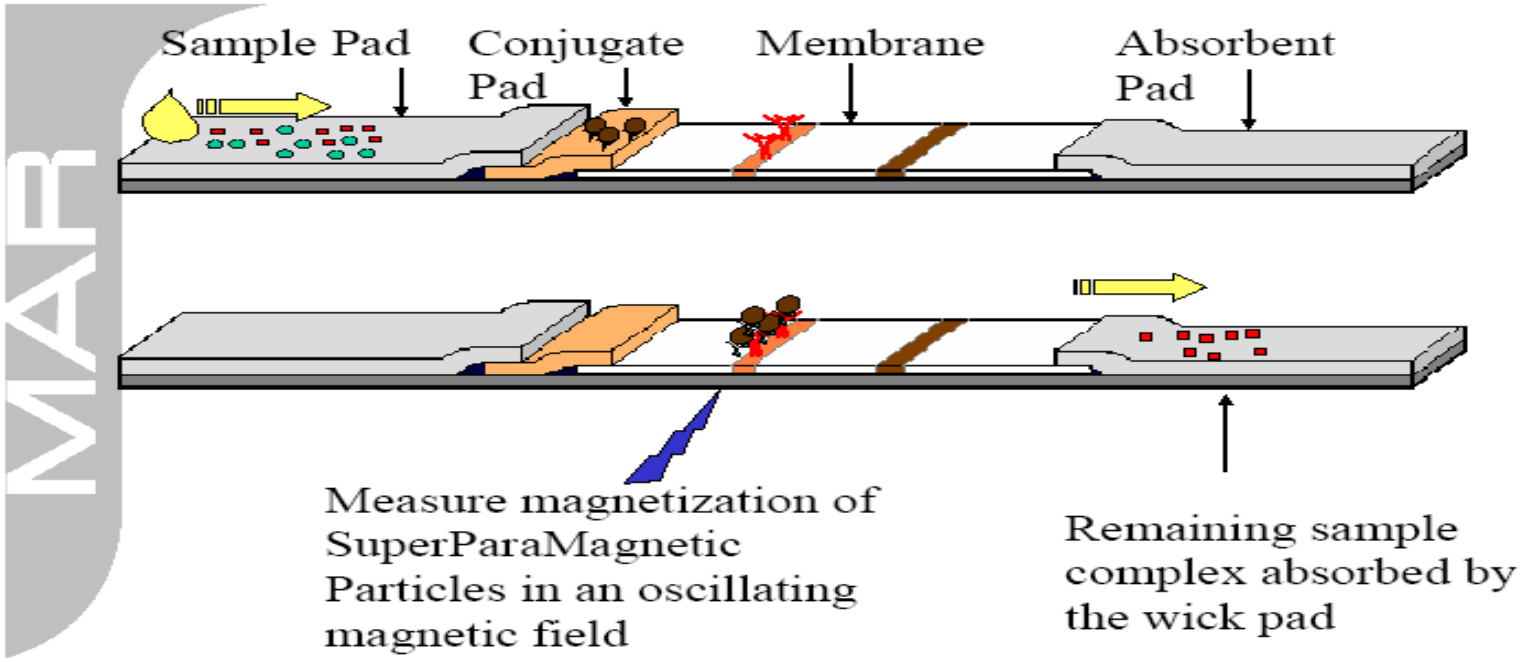
Applications for additional Rapid Testing diagnostics

- Blood safety
- Improved surveillance
- Identification of primary HIV infection
- Monitoring anti-viral therapy
- Diagnosis of infected newborns

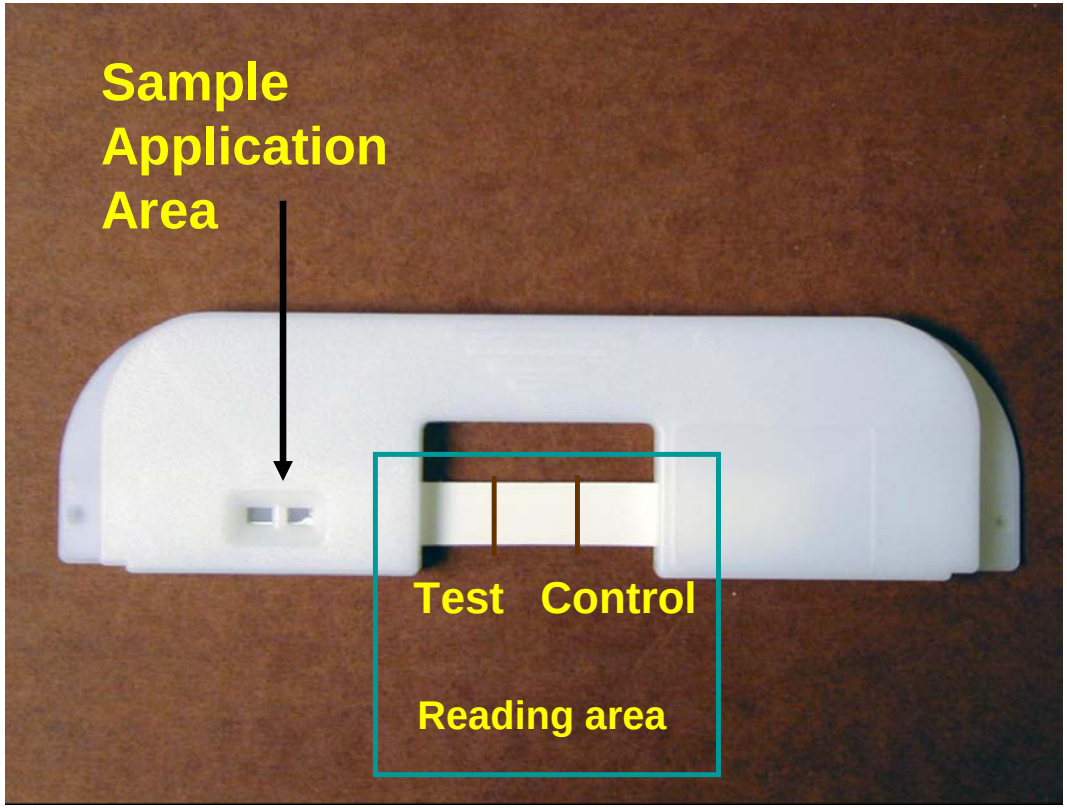
Magnetic immunochromatography (MICT) MagnaBioSciences, LLC

1. **Lateral flow format**
2. **Uses super-paramagnetic beads as test marker**
3. **Detects all captured target at test line improving sensitivity**
4. **Simple to perform**
5. **Cost / test is comparable to current lateral flow tests**

Rapid Diagnostics
MBS MICT assay

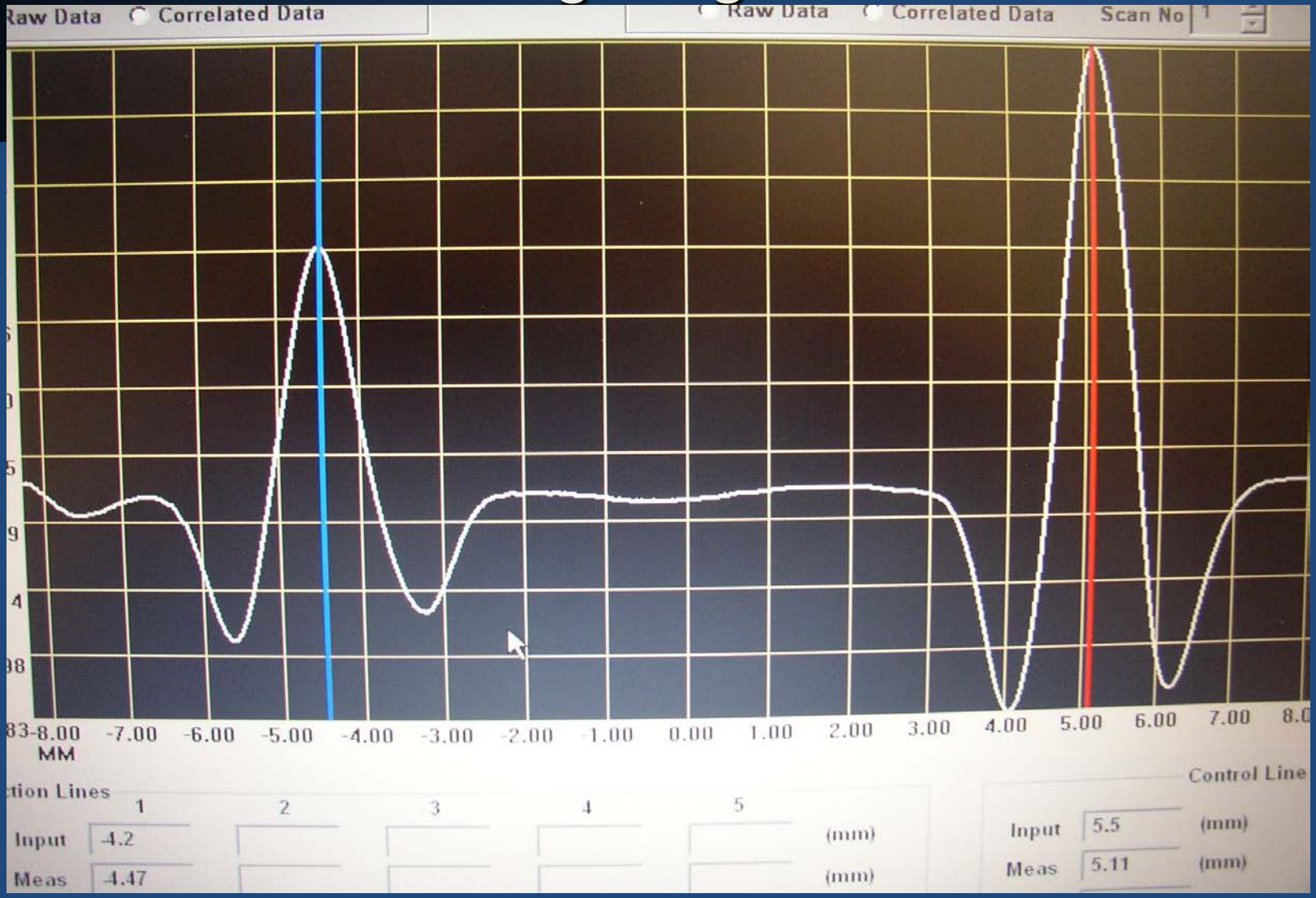


MICT Assay


Sample
Application
Area
Test
Control
Reading area

MagnaBioSciences detection instrument – CE certified

Detection of magnetic field distortion in oscillating magnetic field



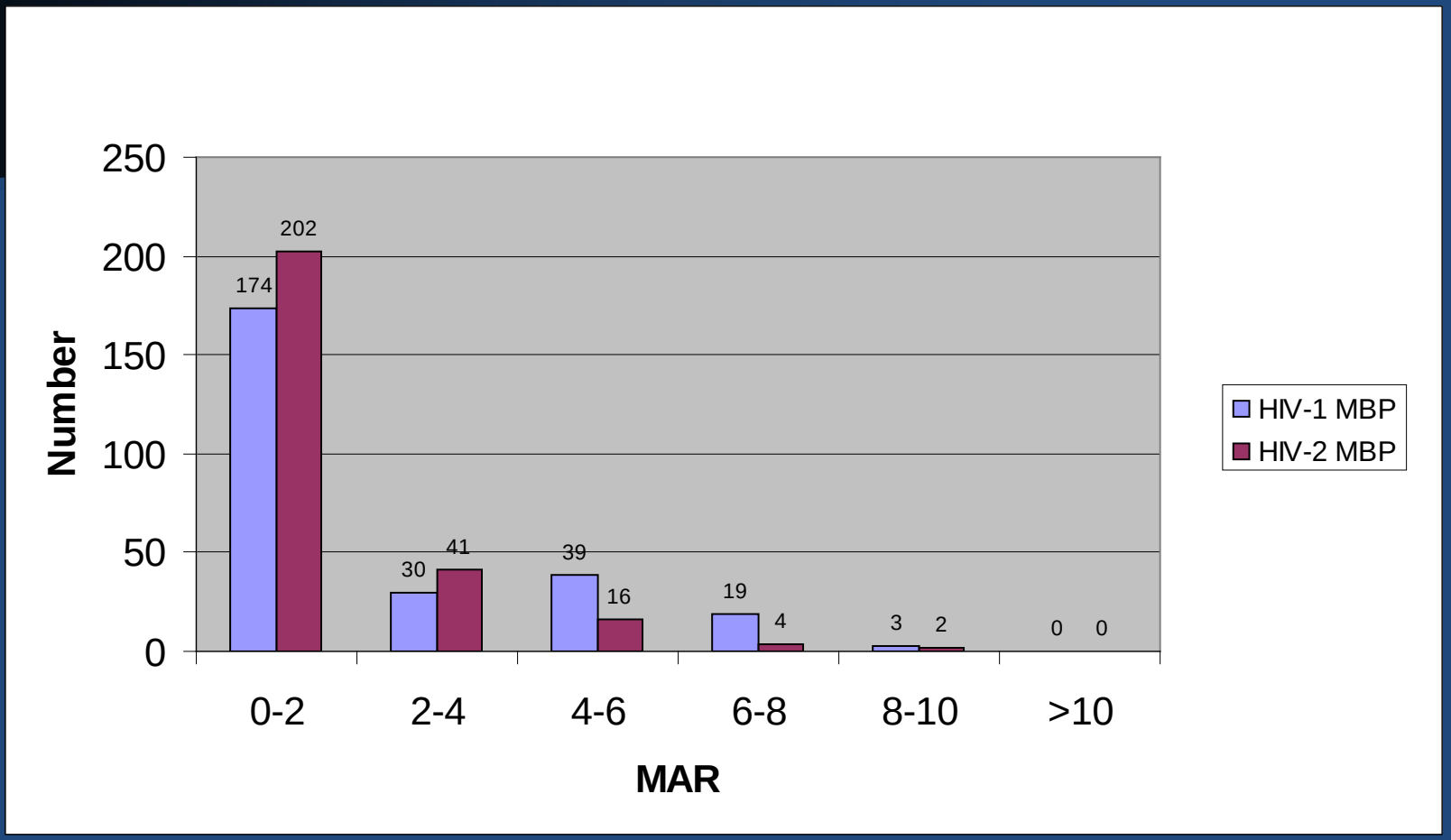
Computer software validates the data and derives a normalized numeric value (MAR)

Detection and differentiation of antibodies to HIV-1 and HIV-2

- Antigens –
  - Protein A as control
  - eight-branched peptide including multiple immuno-dominant regions of HIV-1
  - eight branched peptide for HIV-2
- Protein A/300nm paramagnetic particles
- Dilution of 1:100
- 20 min incubation

Typical MAR data for HIV antibody-positive and antibody-negative specimens
Indirect immunoassay – 2nd generation

		MAR @ 20 min	
	Specimen	HIV-1	HIV-2
HIV-1	1	535.8	0
	2	750.6	0
	3	1009.5	0
HIV-2	4	0	1054.8
	5	0	412.0
	6	0	1531.5



MAR values for HIV antibody non-reactive specimens (N=265)
on the multi-branched peptide (MBP) antigen lines for HIV-1 and HIV-2.

Detection of antibodies to HIV 1 & 2 by MICT compared to detection of HIV-1 and HIV-2 antibodies by EIA/WB reference standard. Specimens from US, west and central Africa; N=549

Indirect immunoassay

EIA/WB Result	MICT		
	HIV-1 P	HIV-2 P	HIV N
HIV-1 P	134	0	0
HIV-2 P	0	65	0
N	0	0	350

Sensitivity (%)	100 %
Specificity (%)	100 %

HIV-1/2 antibody detection by MICT

- Detection of HIV-1 seroconversion 13 panels equal to or better that WB but not as good as 3rd generation EIA
- HIV-1 subtypes were detectable by the MICT assay using specimens from global locales and commercial sources.
- Would an antibody sandwich assay improve sensitivity?
- Only modification required is the paramagnetic conjugate

Typical MAR data for HIV antibody-positive and antibody-negative specimens
Antibody sandwich immunoassay – 3rd generation

		MAR @ 20 min	
	Specimen	HIV-1	HIV-2
HIV-1	1	723.3	0
	2	873.7	0
	3	964.6	0
HIV-2	4	0	676.4
	5	0	436.5
	6	0	368.1

Detection of antibodies to HIV 1 & 2 by MICT compared to detection of HIV-1 and HIV-2 antibodies by the EIA/WB reference standard. Specimens from US, west and central Africa; N=237

Antibody sandwich immunoassay

EIA/WB Result	MICT		
	HIV-1 P	HIV-2 P	HIV N
HIV-1 P	117	5	0
HIV-2 P	0	91	0
N	0	1	28

5 HIV-1 SPECIMENS WERE RX ON HIV-2 AGN;
NO HIV-2 SPECMENS WERE RX ON HIV-1 AGN

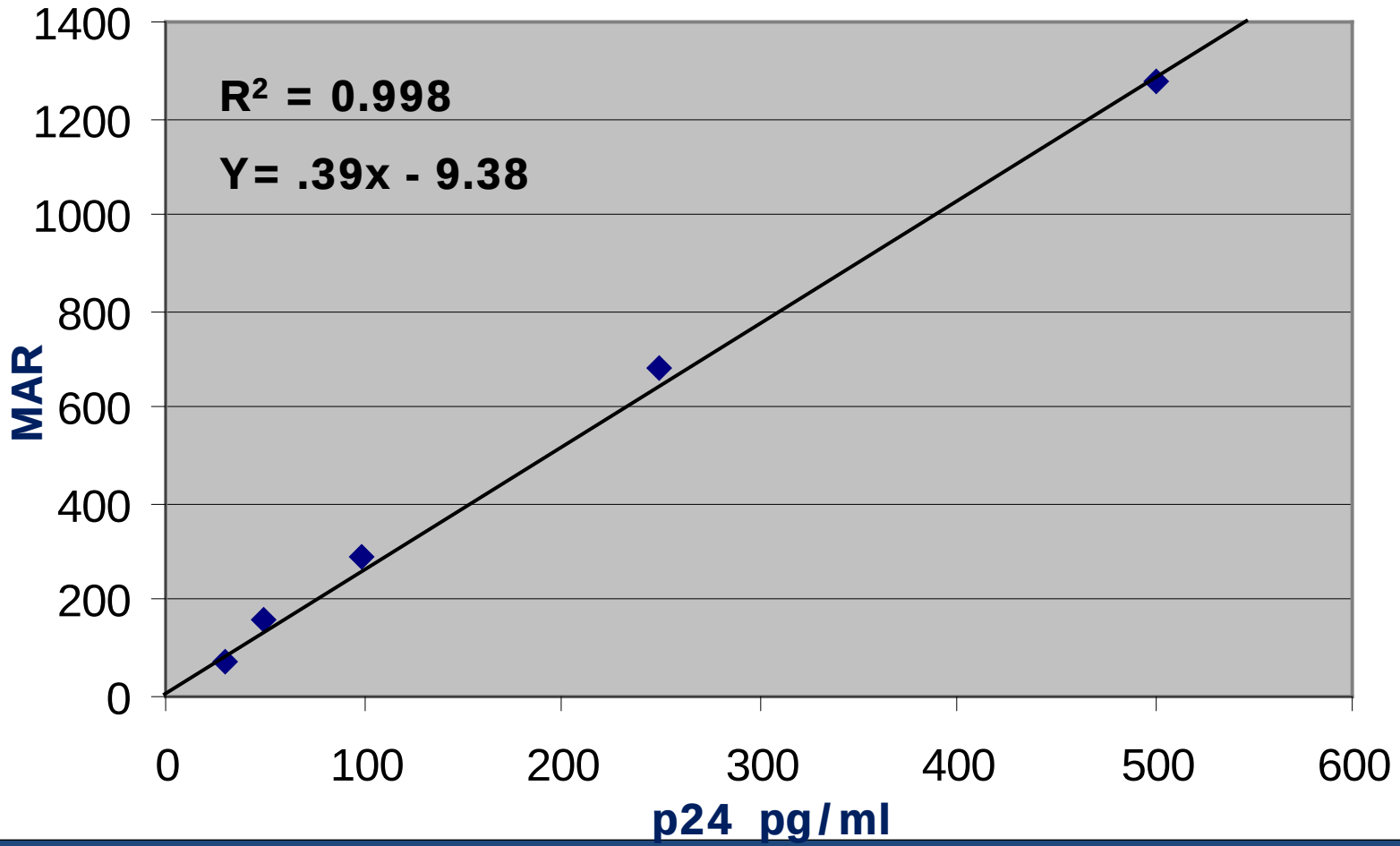
Detection of HIV-1 p24
Challenges

- Concentration of p24 (low pg/ml LOD)
- Availability of analyte (Ag/Ab complexes)
- Unique reagents required
- Assay optimization
- Plasma matrix effects

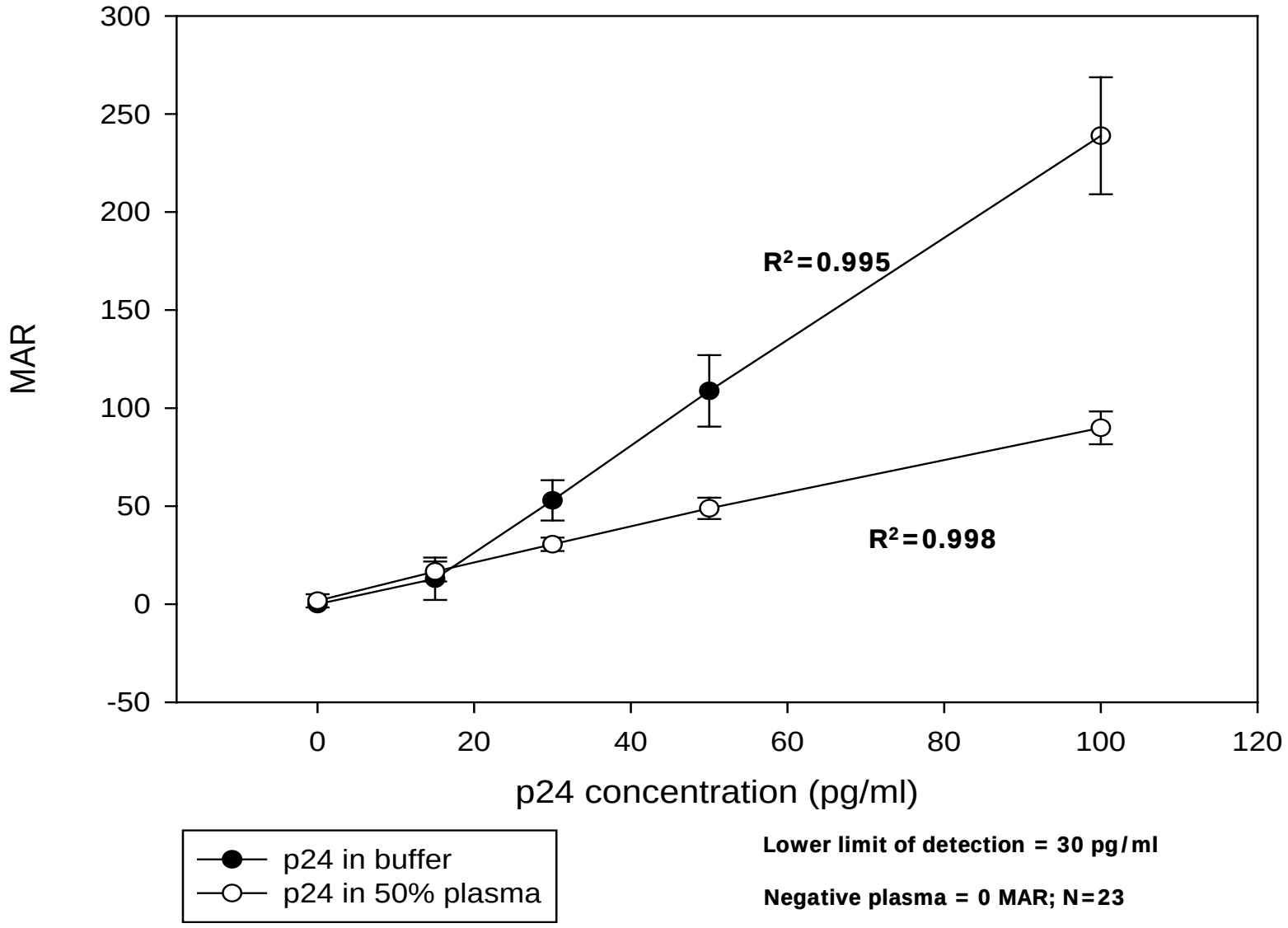
Detection of HIV-1 p24 by MICT

- Rabbit polyclonal capture antibody
- Mouse monoclonal conjugate
- Running buffer selection
- Assay optimization parameters
  - Incubations, reagent concentrations, materials

Detection of HIV-1 p24 spiked into running buffer



Detection of HIV-1 p24 in sample buffer and 50% plasma



Lower limit of detection = 30 pg/ml

Negative plasma = 0 MAR; N=23

Detection of p24 antigen in seroconversion panels by MICT

Panel	Member	Day	EIA S/CO	WB	VL	p24 S/CO	MICT (MAR)
1	1	14	0.1	N	$2x10^3$	0.2	0
	2	16	0.1	N	$9x10^4$	1.5	13.4
	3	21	0.1	N	$>8x10^5$	>50	46.4
	4	23	0.4	N	$>8x10^5$	>50	681.0
	5	103	17.9	P	$2x10^4$	0.1	0
2	1	7	0.1	N	$1x10^5$	1.5	1.5
	2	12	0.2	N	$>8x10^5$	32.3	29.0
	3	14	2.5	N	$>8x10^5$	>44.0	111.3
	4	19	>18.2	N	$>8x10^5$	27.6	42.6
	5	21	>18.2	I	$6x10^5$	16.4	20.7

HIV-1 p24 detection

- Detection of HIV-1 p24 antigen in various HIV-1 subtype culture fluids
- Detection of p24 antigen in spiked plasma specimens with ~250,000 copies of HIV-1 RNA
- Inter-run and intra-run reproducibility was excellent with CVs <12%

Workman et al. 2009 JVM 160:14-21

Detection of HIV-1/2 antibodies and p24 antigen by MICT

Panel member	Abbott HIV 1/2 s/co	Abbott p24 agn s/co	Roche PCR	WB_Band Pattern	2nd gen MICT	3rd gen MICT	p24 MICT
AC-01	0.2	0.6	NEG	no Bands	1.5	0	0
AC-02	7.8	>22.7	POS	no Bands	0	0	177.6
AC-03	15.1	2.2	POS	24,55,160	90.9	982.8	0
AC-04	15.6	1.8	POS	24,55,120,160	151.1	794.3	0
AC-05	16.4	1.0	POS	18,24,55,120,160	146.5	1106	0

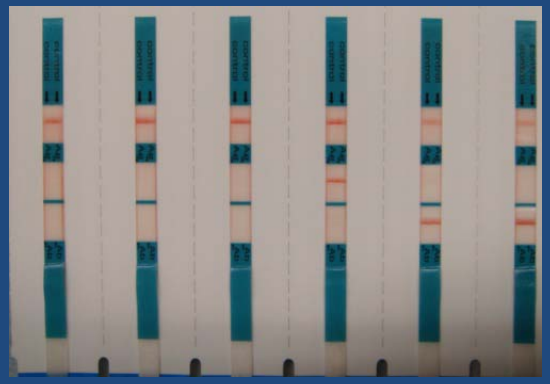
Status of MICT based assays Next steps

- Reproducible detection of 30 pg/ml in plasma
- Detection of spiked virus at $2.5X10^5$ virions/ml
- Detection of p24 in seroconversion panels
- Detection of p24 in culture supernatants from various HIV clades (A-G, O)
- Second and third generation aby tests have been developed

- Aby/Ag combination rapid test

Applications for additional Rapid Testing diagnostics

- Detection of HIV-1 p24 in physiologically relevant ranges is feasible
- Rapid p24 testing will be incorporated into more assays
- Applications for p24 testing to be established
- Low-tech readers for objective determinations



Acknowledgements

- CDC
  - Shon Workman
  - Ae Youngpairoj
  - Michele Owen
  - Susan Wells
  - Chou Pau
  - Angela Holder

SRP/CDC
- John Hart
- Kelly Jeter
- James Partin

MagnaBioSciences
- Jim Wyatt
- Ron LaBorde
- Fan Dong
- Dave Pratt